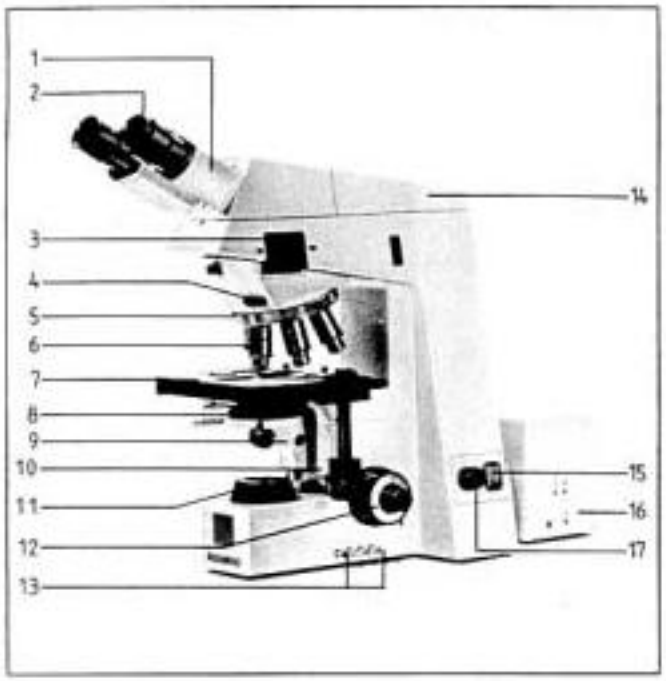
ZEISS

Axioskop

Routine microscope

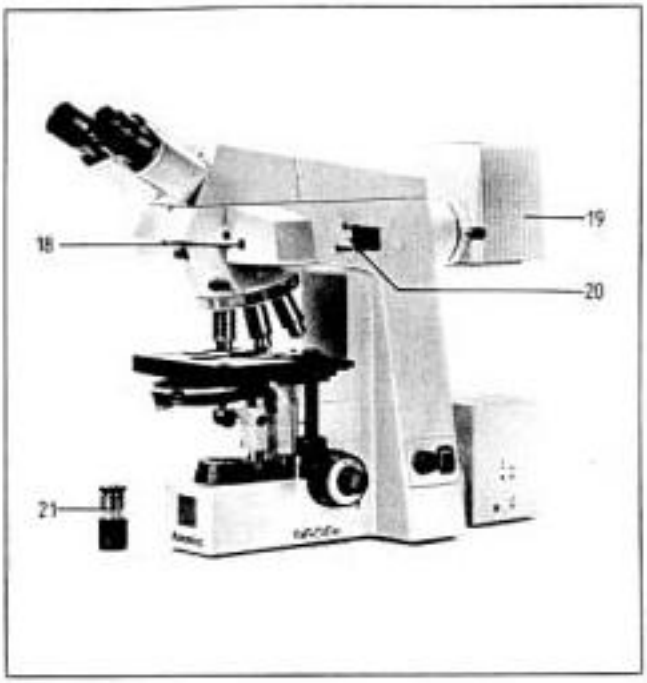
for transmitted light and incident-light fluorescence

Operating manual



Axioskop routine microscope for transmitted light

1 Binocular tube
2 Eyepieces
3 Slot for Optovar slider and reflector slider FI
4 Slot for auxiliary objects and analyzer
5 Nosepiece
6 Objective
7 Specimen stage
8 Condenser
9 Condenser carrier
10 Stage carrier
11 Luminous field diaphragm



Axioskop routine microscope for transmitted light and incident light fluorescence

12 Coaxial coarse/fine focusing control
13 Filter magazine
14 Tube panel
15 ON/OFF switch with signal lamp
16 Transmitted-light illuminator
17 Potentiometer for Hal illuminator
18 Reflector slider 3 FI
19 Incident-light fluorescence illuminator
20 Incident-light system FI
21 Centering telescope with 30mm dia.

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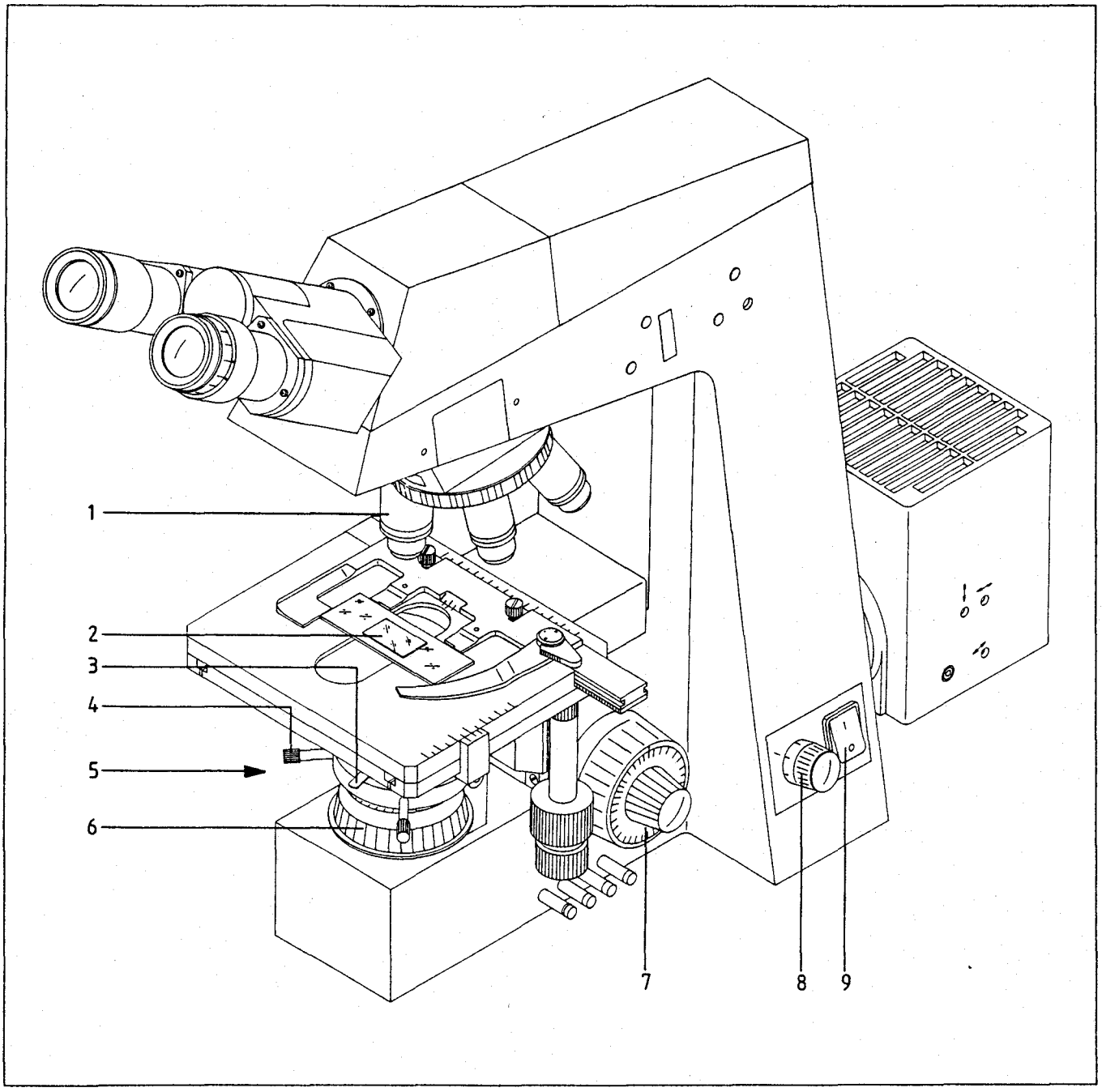
Special notes

- The 6- to 10-digit numbers, e.g. 45 14 55, are ordering numbers of instruments or components.
- Changes and/or repairs of the instruments should be carried out only by the manufacturer or his authorized representative.
- Specifications subject to change.
- **Caution!** Do not operate the instruments in explosion-risk areas.

1



Special note: Bold numbers like **1.1** refer to the detailed description of the instrument starting on page 6.

- Check whether the data on the power rating plate (instrument back) comply with your local power data. Connect microscope to the line with power cable, switch on with (9) and set to 3 - 4V with (8).
(Axioskop FS: plug the Hal illuminator cable into power supply unit (39 25 85), connect it to the line and set the brightness required with the potentiometer, see page 9.)

- Load a high-contrast specimen (2) on stage (smaller, thin cover glass face up).

- Turn in a 10x objective (yellow ring) (1), check the 0-positions on the eyepiece scale, and use (5) to move the condenser up until stop (front lens not swung out).

- Set index of condenser turret to H (brightfield) and close the condenser diaphragm about half (3).

You should now see light spots (the exit pupils) behind the eyepieces. If you use a binocular phototube with sliding prism, push in the pushrod.

When looking into the tube you will see a bright circle with each eye (the eyepiece diaphragm). Setting the two eyepiece tubes to your PD will merge the two circles into one.

For adjustment of Köhler illumination proceed as follows:

- Focus the specimen with coarse/fine focusing control (7). (If your eyes have different powers or if you work without eyeglasses → **6.5.**)

- Close luminous field diaphragm (6) moderately; its contours will be blurred (A).

- Focus the image of the diaphragm by slightly lowering the condenser with (5) (B).

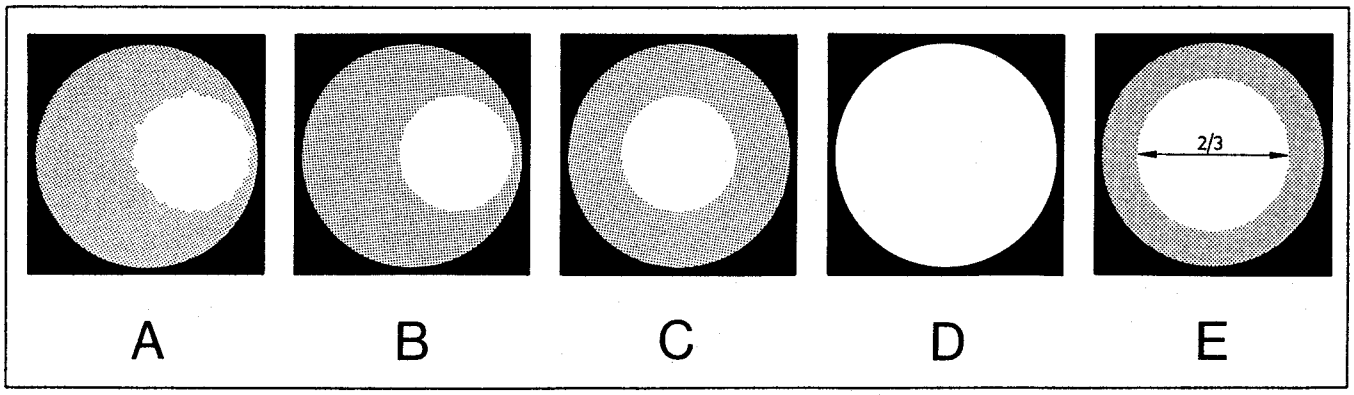
- Use screws (4) to move the diaphragm image to the center of the field of view (C), and

- open luminous field diaphragm (6) until it just disapears from the field of view (D).

Use condenser diaphragm (3) to adjust the contrast individually for each specimen. If you are not certain how far to stop down: approx. two third of the rear element of the objective (visible at the bottom of the tube if the eyepiece is removed from the tube) should be illuminated (E) if a specimen is of moderate contrast. The field of view and the objective aperture change with objective exchange. Always repeat the last-mentioned steps when you exchange objectives.

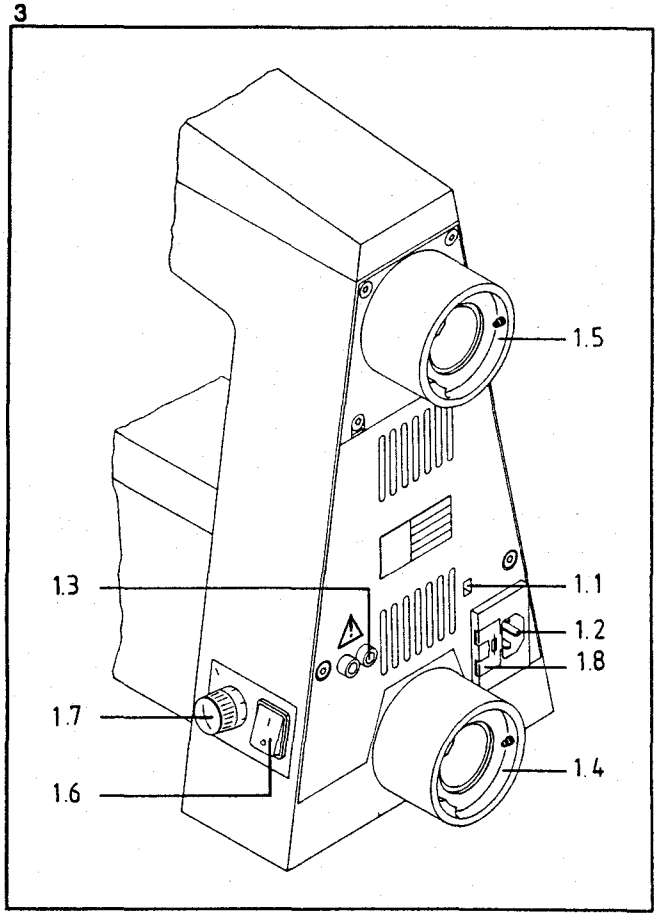
The condenser front lens must be swung out when a low-power objective images more than the condenser can illuminate. For more details see page 11.

2



Special note
Almost all screws you need are Allen screws for which we supply:
SW 3 and SW 1.5 Allen wrenches with red handle. They are needed to swing the diffusing screen in or out, and to mount tubes, illuminators and the dust covers.
The stability of the microscope can be increased using a microscope base plate, e.g. (45 14 93).
For mounting, remove tube, tube panel and both pins. Turn the stand upside down, unscrew 4 rubber feet and place the 4 spacers supplied onto threaded holes. Attach microscope base plate (upper side pointing downwards) and screw it to stand using 4 screws supplied. Attach tube and tube panel again.

3



1.0 Lamp power supply

The power supply for the Hal illuminator is integrated in the stand (not in Axioskop FS).

1.1 Voltage dial indicating the internally adjusted input voltage either for 230V (220 . . . 240V ~) or 115V (110 . . . 120V ~), frequency is 50 . . . 60Hz in both cases.

If the local voltage does not lie within the adjusted voltage range, the selector can be reset vertically using a screwdriver; for this, the instrument must be switched off first and the cable disconnected from the line.

1.2 Instrument plug with integral fuses for 230V or 115V, depending on the value set on the voltage dial. If the dust cover **1.8** is swung aside the fuses are accessible for exchange (see p.31).

⚠ Connect only the lamp housing (44 72 17) with 12V 50W halogen lamp (38 00 79 - 9550) to the sockets **1.3** .

1.4 Port for attachment of transmitted-light illuminator. It contains a 42mm dia. CB 3 conversion filter.

1.5 Port for attachment of incident-light illuminator. In fluorescence microscopy, the 42mm dia. holder does not contain a filter.

1.6 ON/OFF switch and signal lamp.

1.7 Potentiometer supplying maximum voltage when turned fully clockwise. The adjusted voltage can be read off on the index.

2.0 Illuminator Hal

The standard equipment includes the lamp housing Hal (44 72 17- 9901) with reflector, collector, heat-reflecting filter and mount, and a 12V 50W halogen lamp (38 00 79-9550). Connection to sockets **1.3**.

2.1 Light exit port with dovetail ring for mounting on the microscope:
- Loosen screw at **1.4** or **1.5** as far as required.
- Attach dovetail ring of illuminator in recess opposite the clamping screw in an inclined position, lower illuminator on to the seating surface and tighten the screw.

A holder in the light exit in front of the collector contains a 42 mm dia. heat-reflecting filter. If the illuminator is used for UV blue fluorescence excitation, this holder must be empty.

2.2 Clamping screw to secure the lamp housing on the lamp socket.
2.3 Focusing of lamp coil
2.4 Vertical adjustment of lamp coil
2.5 Horizontal adjustment of lamp coil

Note: The lamp change is described in the operating instructions G 42-216 for the Hal illuminator with reflector.

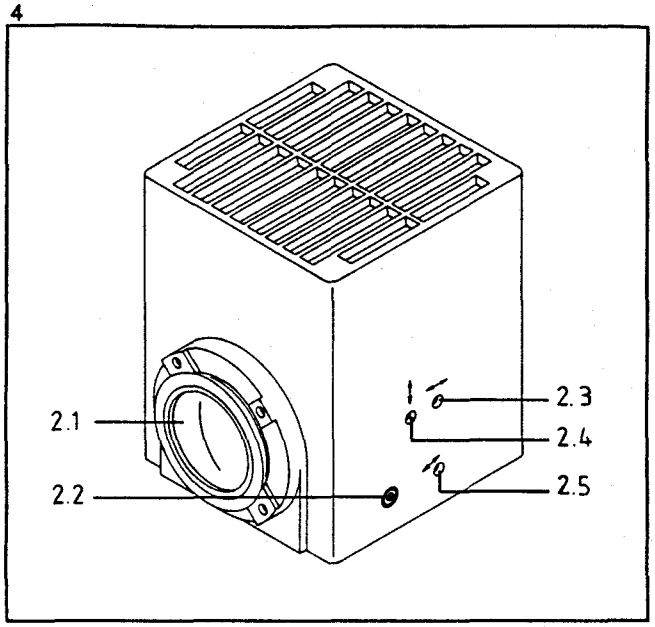
Adjustment of halogen lamp with reflector

- Remove illuminator Hal from microscope.
- Switch on halogen lamp via power supply.
- Use screw **2.3** to project coil image on a wall or similar object approx. 3 m away.
- Use screw **2.4** for vertical adjustment of the coil image and screw **2.5** for horizontal adjustment. Move the coil image in such a way that the gaps of the reflector image are covered (see Fig. 5).

The fixed reflector need not be adjusted, since it lies exactly in the optical axis.
Fine adjustment:
- Remove internal tube with diffusion disk **3.2** (turn socket head screw counterclockwise to bright circle) and filter, if used, from light path. Attach microscope illuminator and focus on specimen using an objective with magnification 40x or higher. Then find a vacant specimen position.
- When the eyepiece is removed or the centering telescope inserted into the tube, check whether coil image and reflector image are in center position. If not, make correction using the adjusting screws mentioned above.
- Replace diffusion disk **3.2** and filter into the light path again.

Check homogeneous illumination of the pupil image and correct it, if required, using screw **2.3**.

4



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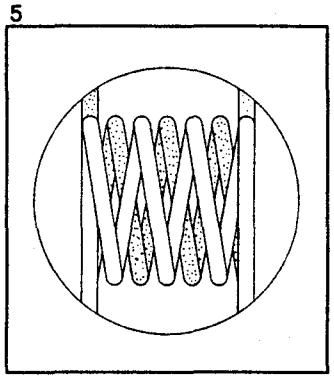
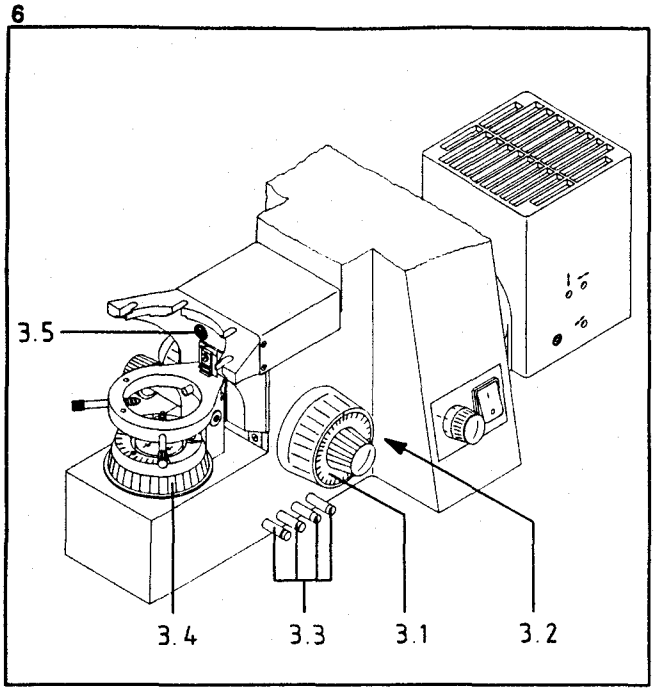


The lamp housing HBO/XBO with HBO 50 mercury short arc lamp, which is described on page 19, is required for incident-light fluorescence.

6



3.0 Stand bases

3.1 Coaxial coarse/fine focusing control. It acts on the stage and condenser carrier which is an integral part of the stand. Turning the outer part of the knob towards the user lowers the stage. Overall travel (including fine focusing control): 25mm. One turn of the coarse focusing control corresponds to 2mm travel; the gear ratio of the fine focusing control is 1:10. The index line on the coarse focusing control can be used to roughly measure the specimen thickness: 1 interval corresponds to approx 2 µm.

3.2 Diffusing screen. Turning it with a wrench to the dark circle produces homogeneous illumination of the pupil.

3.3 Filter magazine in illuminating beam path with 4 pushbuttons (from front to back):

- Dark-gray ring: 32mm dia. neutral density filter 0.06.
- Light-gray ring: neutral density filter 0.25
- Green ring: interference green filter
- Blue ring: conversion filter 3200/5500 K

The neutral density filters used singly or in sets serve to vary the brightness in steps. The transmittance of a filter set is determined by multiplication
(e.g. 0.06 x 0.25 = 0.015,
i.e. 1.5% transmittance).

A green interference filter enhances the contrast for B/W photography and phase contrast.

A conversion filter converts artificial light of 3200 K into daylight of 5500 K.

If several filters are to be used at a time, the corresponding pushbuttons must be pressed simultaneously. The filters are removed from the beam path if the foremost button is pressed.

The exchange of filters in the magazine should be carried out by a service technician. (The bottom plate must be removed. A filter secured by a retaining ring becomes accessible if the remaining filters are swung in).

3.4 Luminous field diaphragm. It is adjusted with the knurled ring. The (removable) dust cover glass accepts a 32mm dia. filter. This plane is not imaged.

3.5 The eccentric disk limits the height adjustment of the condenser. The height stop is factory-aligned, but can be changed using a screwdriver, if required.

Axioskop FS stand (45 14 06)

The microscope Axioskop FS (FS = fixed stage) features objective focusing instead of stage focusing. The nosepiece H DIC can be moved by 25 mm. Its lower stop position corresponds to the focus position of the standard Axioskop stand.
Please note the following **differences**, however:

- Remove transport locking screw at **3.6**, marked red, on back of the stand before operating focusing controls **3.1**.
Store the locking screw so that you can use it when the instrument is transported to another location again. To insert the screw again, turn the coarse control until the threaded hole in the stand coincides with the hole at the back of the stand.

To lift the microsocpe Axioskop FS, always grasp it at the stand base and not at the top.

- The stage carrier can be lowered by 17 mm after the two screws **3.7** have been loosened.

- The microscope illuminator Hal (44 72 18-9901) can be used with 12V 50W halogen bulb (38 00 79-9550) or with 12V 100W halogen bulb (38 00 79-9540). It is connected to the stabilized power supply unit (39 25 85). Check whether the correct voltage has been set, connect the power supply to the mains, switch on the lamp via knob and select the required brightness. The set voltage can be read off at the scaled ring.

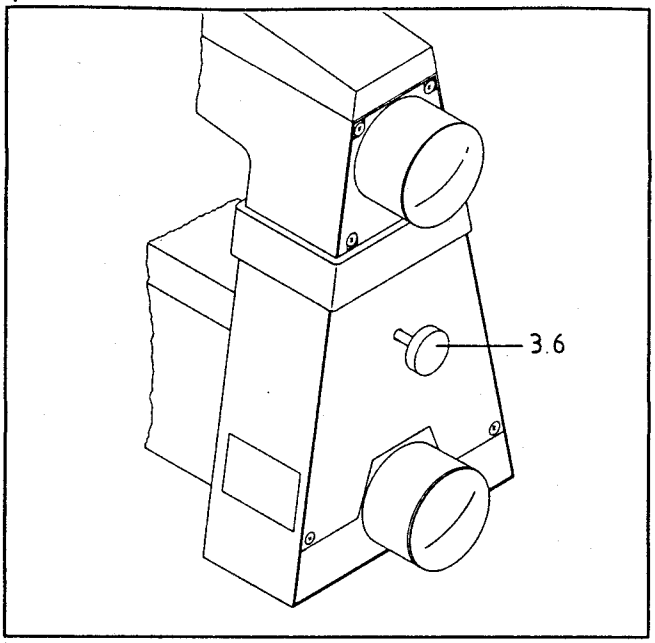
The power consumption is 170 VA. The frequency is 50 - 60 Hz. The input voltage is pre-adjusted for 100 - 110 - 120 - 127 - 220 or 240 V AC, and indicated on the back side of the power supply unit, which is radio-screened and in compliance with VDE, IEC, CSA and UL regulations. The power supply unit is categorized as a safety class I, type B instrument.

- Stop **3.9** limits the vertical travel of the condenser (max. 32 mm) and prevents unintentional pressing out of the specimen by the condenser. The stop can be set as follows: focus on specimen, image luminous field diaphragm. Loosen screw **3.8** with key supplied. Move condenser slightly upwards (diaphragm image is slightly out of focus). Tighten screw to fix stop pin.

When inserting the condenser, make sure that the pins 3.10 of the condsenser mount engage with the slanted grooves of the condenser (Fig. 32). The aperture scale must be aligned symmetrically to the user.

Note: The grounding of the Axioskop FS is described on page 30.

7



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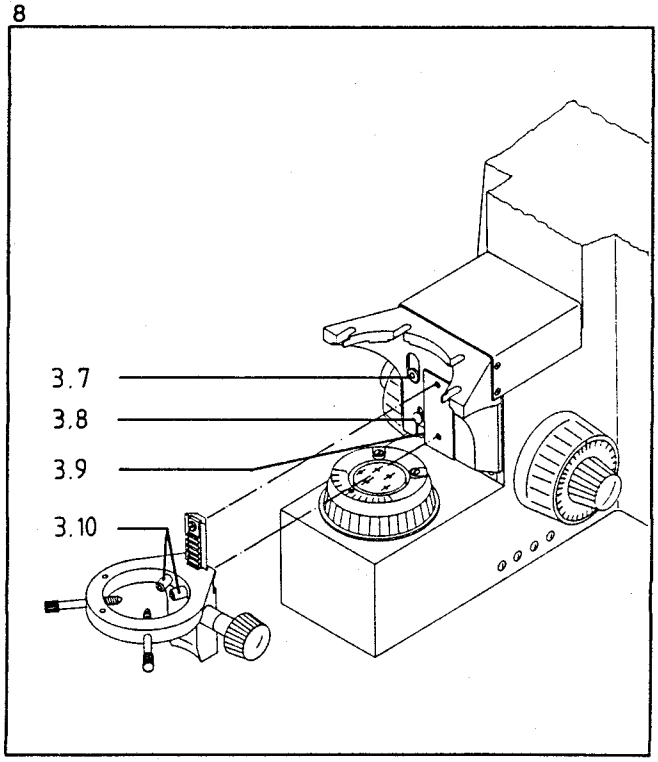

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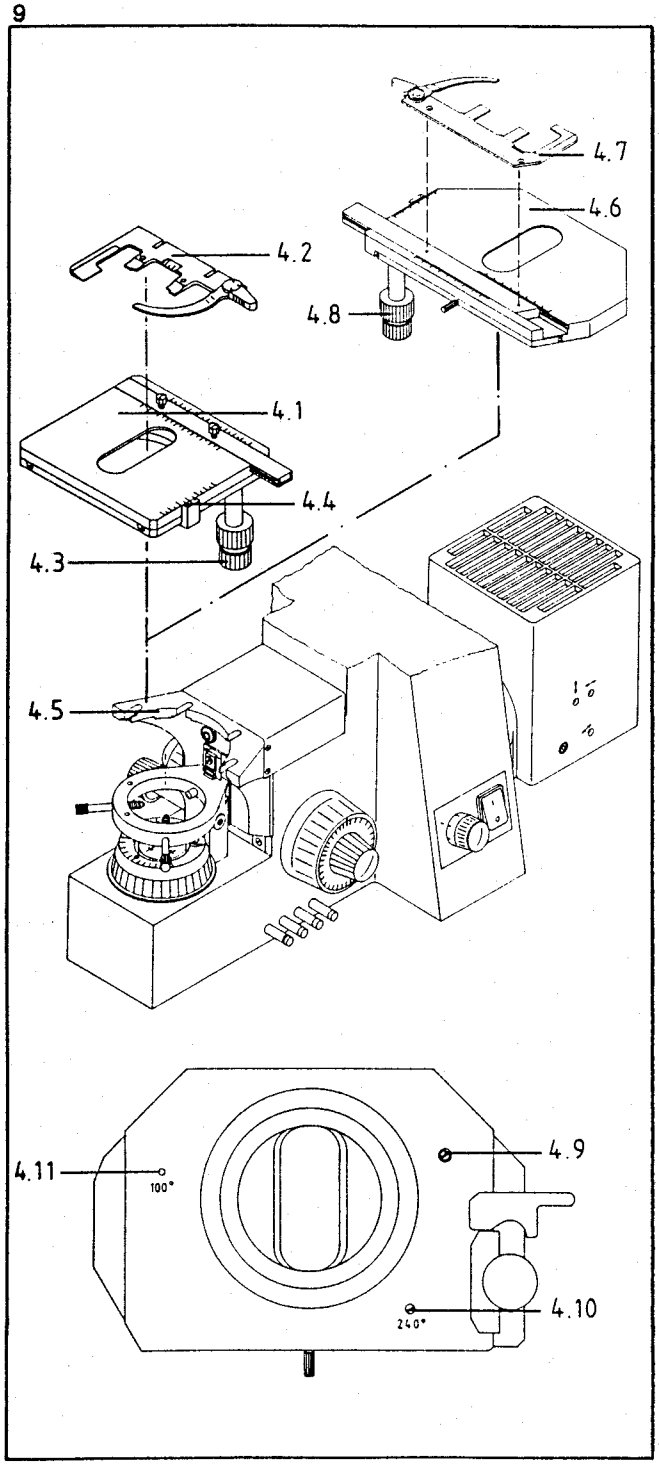


4.0 Specimen stage

Standard outfit includes:

4.1 Mechanical stage 75 x 30 R with low-mounted control, right (45 35 22), with

4.2 Specimen holder with spring clip to the right (473448) for standard microscope slides (76x26 mm) and for those in the format 45x26 mm, or Universal mounting frame for Petri dishes and microscope slides (453545) especially for the Axioskop FS. If the Universal mounting frame is used, the mechanical stage (453522) must be rotated through 180° (stage drive on the front left) for mounting. The stop pin (underside of stage) for the y-travel range must be removed from 30 mm to 50 mm.

4.3 Coaxial controls for 75mm x 30 mm stage travel in x and y.
4.4 Graduations and verniers for the relocation of specimen features.

4.5 Stage carrier with specimen stage secured with 4 screws. The stage should be changed by a service technician only.

4.6 Mechanical stage 75x50R/240° (453503-9901), rotatable through 240°.
Stage size: 150x220 mm
Travel range in x-direction is 75 mm, in y-direction it is limited to 28 mm, but can be extended to 50 mm if the screw 4.9 (marked with a white circle) on the underside of the stage is removed. The 28 mm travel range in y allows the stage to be rotated through 240° without restriction. With the 50 mm range, however, rotation is limited because the stage might hit the stand. To avoid this, the range of rotation can be limited to 100° as follows:

- Turn stage **4.6** until drive **4.8** points to the front (as shown).
- Remove screw **4.10** to the position **4.11**.

4.7 Specimen holder with spring clip (453533) is used.

5.0 Condensers

5.1 Condenser carrier with:

5.2 Knob for vertical adjustment. Its stiffness is adjusted in the factory and should be changed only by a service technician. To mount a condenser H, D, Ph (44 52 36) or Ph, DIC (44 52 41), insert the rear notch in

5.3 spring pin, and the entire dovetail ring mount in the corresponding opening of the condenser, which aligns the condenser in straight forward direction (aperture scale symmetical).

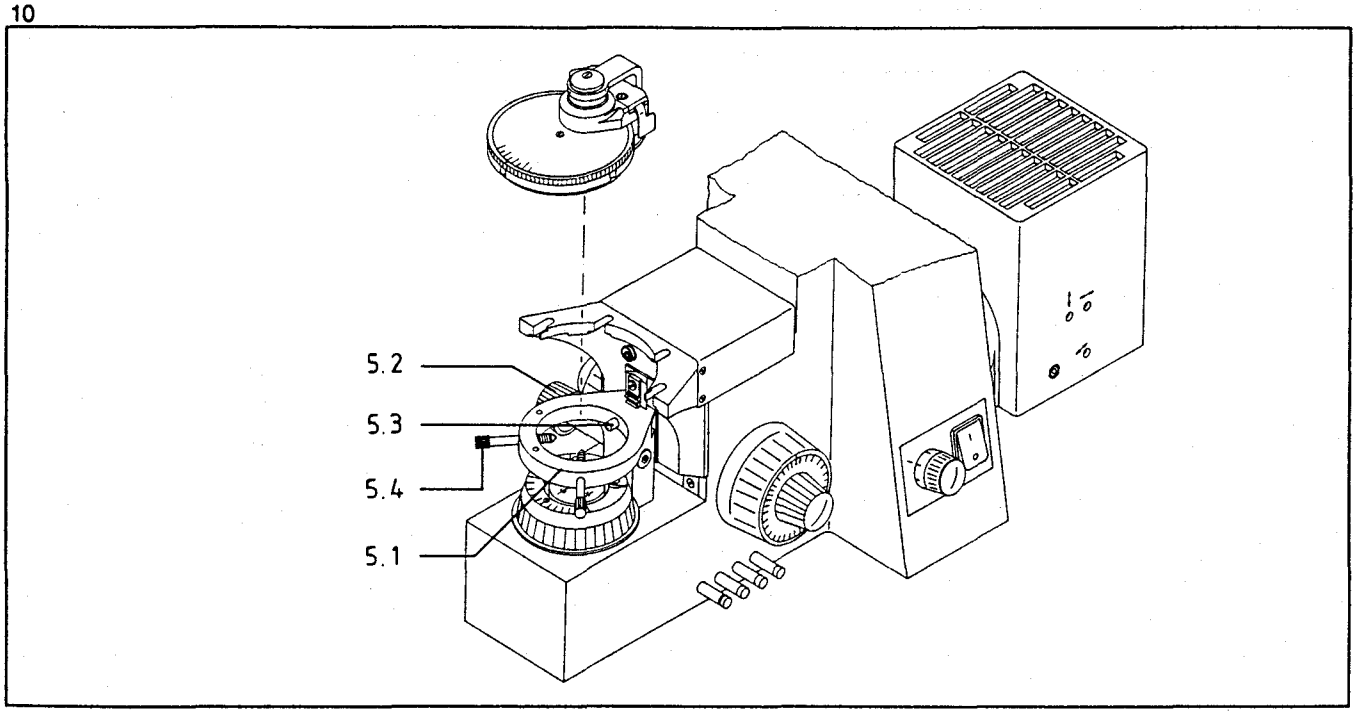
5.4 Two condenser centering screws to center the image of the luminous field diaphragm for illumination adjustment (see page 5).

A swing-in color-glass carrier or a polarizer can be inserted in a holder under the condenser carrier.

A limit stop for the vertical condenser movement prevents a specimen from being pressed out by mistake.
The vertical adjusting range for standard specimens is adjusted in the factory, but may be altered by a service technician.

The working height of the condensers described below is 41mm.

10



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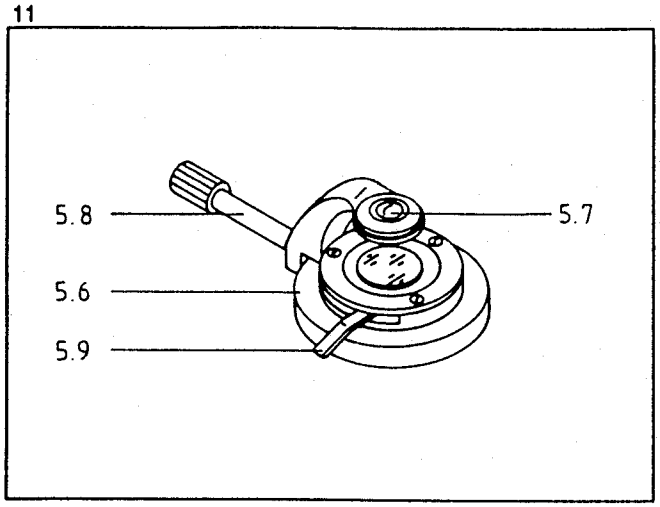


We offer a great number of condenser systems, to meet the high demands that are made on the versatility of a more-than-routine microscope.

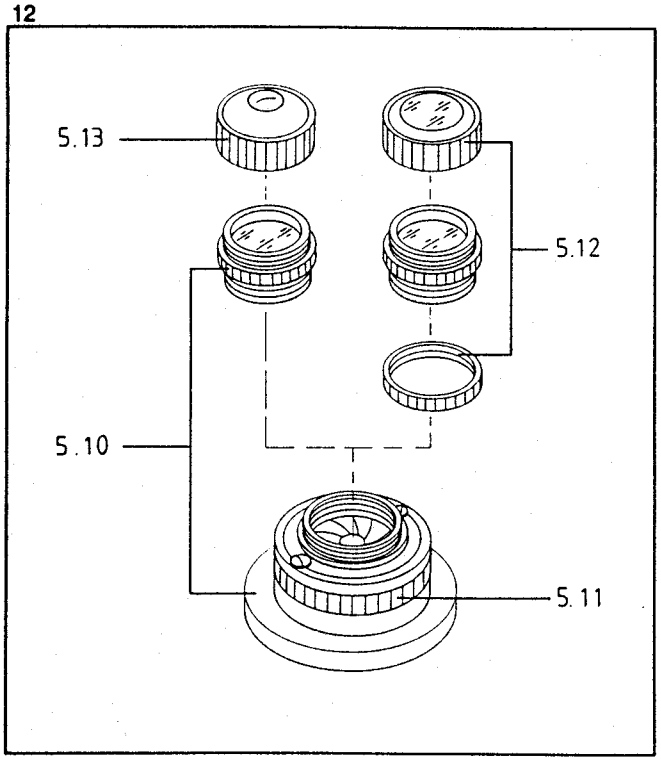
5.6 Brightfield condenser 0.9 Z with swing-in front lens (44 52 30)

5.7 Front lens, aperture 0.9, swung in for Köhler illumination with 5x . . . 100x objectives.

5.8 Lever to swing out the front lens (for 2.5x . . . 4x objectives).

5.9 Lever for aperture iris diaphragm.

12



For long back focal distance or high aperture (1.4) in brightfield or DIC:

5.10 Achromatic-aplanatic condenser system 0.32 Pol (44 52 45-9902) for brightfield, with removable optical system, aperture 0.32, suitable for Köhler illumination with 2.5x . . . 10x objectives.

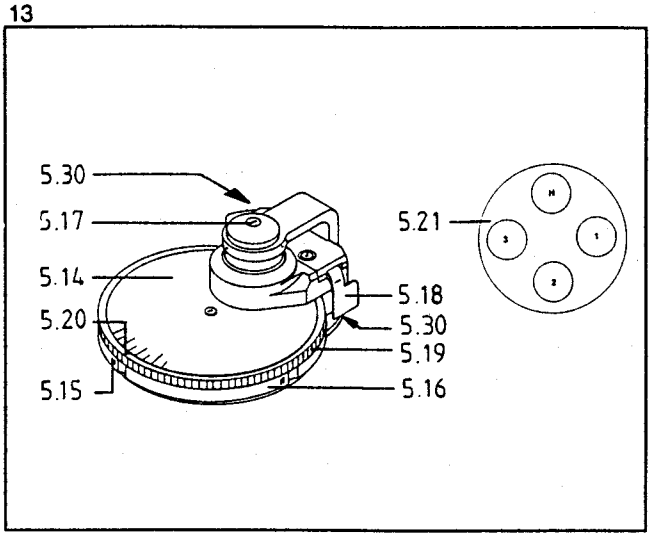
5.11 Knurled ring for aperture iris diaphragm.

The following items can be screwed on the condenser system 0.32 Pol:

5.12 Front lens 0.63 Pol (46 52 65) for long back focal distance (an additional spacer ring is required);
for brightfield and DIC, Köhler illumination with 5x . . . 40x objectives,
DIC with DIC prism 0.3 - 0.4/0.63 (43 44 10) for objectives 10x/0.30, or

5.13 Front lens 1.4 Pol (46 52 68) for brightfield and DIC with highest illuminating aperture, Köhler illumination with 20x . . . 100x objectives,
DIC with DIC prism 0.5 - 1.4 (44 52 94) for objectives with apertures ≥0.5.

13



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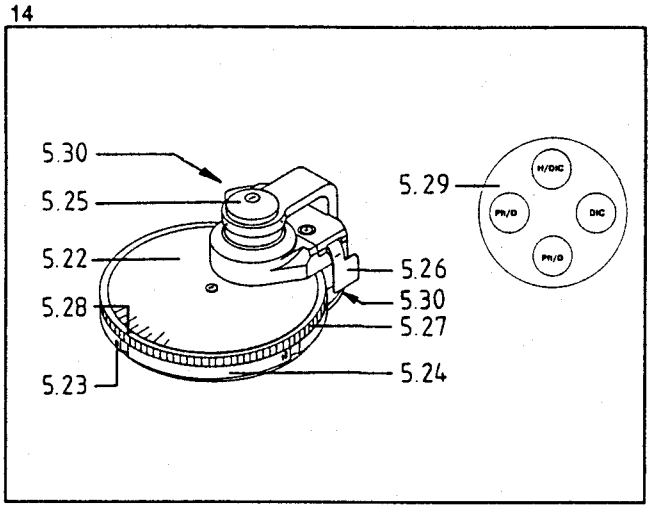


Turret condensers with several click-stop positions allow quick change between illumination and contrast-enhancement methods.

5.14 Condenser 0.9 HD Ph with swing-in front lens (44 52 36), with index **5.15** showing the adjusted turret position.
5.16 Standard turret with apertures for
- brightfield (H)
- phase contrast 1 (no. 1 in green)
- phase contrast 2 (no. 2 in green)
- phase contrast 3 (no. 3 in green)

A darkfield diaphragm (44 52 99), illuminating aperture 0.75 - 0.90, for objectives ≤ 40x/0.75, or auxiliary lens 1.25x (44 52 65) can be inserted in either of the three centerable click-stop positions for phase contrast.
Diaphragms and auxiliary lens are centerable at **5.30**.

5.17 Front lens, aperture 0.9.
5.18 Lever (on both sides) to swing the front lens in or out (for 2.5x and 5x objectives).
5.19 Knurled ring for aperture diaphragm
5.20 Index for aperture reading
5.21 The turret is equipped in such a way that the index in front displays the activated position.

5.22 Achromatic-aplanatic condenser 0.9 Ph DIC (44 52 41) with swing-in front lens
for brightfield, darkfield, phase contrast and DIC.

5.23 Index displaying the activated illumination technique.

5.24 Turret with two centerable apertures for
phase contrast stop Ph 1/0.9 (44 52 69)
phase contrast stop Ph 2/0.9 (44 52 70)
phase contrast stop Ph 3/0.9 (44 52 71)
darkfield diaphragm D (44 52 99) for objectives with apertures ≤0.75,
auxiliary lens 1.25x (44 52 65) for illumination of the object field with 1.25x objective;

two apertures with pre-centered iris diaphragms
for brightfield illumination (H), with holders for
DIC prism 0.3 - 0.4 (44 52 73) for objective apertures 0.3 . . . 0.4
DIC prism 0.5 -1.4 (44 52 74) for objective apertures 0.5 . . . 1.4

5.25 Front lens with aperture 0.9
5.26 Lever (on both sides) to swing the front lens in or out

5.27 Knurled ring for operation of the aperture diaphragm
5.28 Index for aperture reading
5.29 Occupied turret apertures seen from above

The reading indices 1 and 2 do not necessarily refer to built-in Ph 1 and Ph 2 phase stops. A Ph 3 phase stop, a darkfield diaphragm D or an auxiliary lens 1.25x may be included instead, and the numbers then serve merely as guidance.
The H/DIC position must only be equipped if 2 DIC prisms are used. Brightfield is then produced by swinging out a polarizing filter.

It is possible to adjust Köhler illumination
with swung-in front lens with 10x . . . 100x objectives;
with swung-out front lens the condenser is not lowered and illuminates the object fields of 2.5x . . . 5x objectives;
with auxiliary lens 1.25x with 1.25 . . . 5x objectives without lowering the condenser. The luminous field iris diaphragm may be used for contrast enhancement.

For a list of darkfield condensers see page 22.

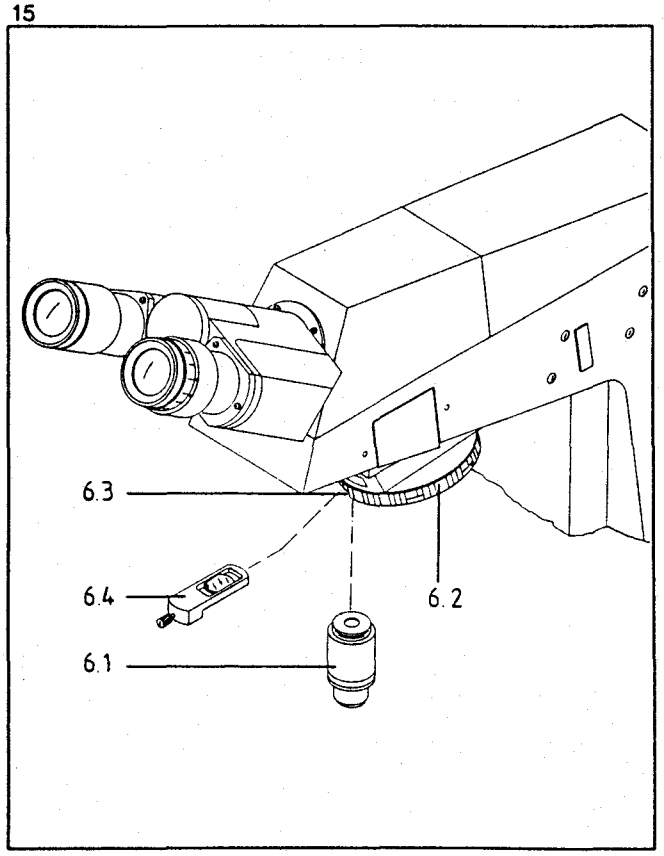
6.0 Image-forming components

6.1 Objectives are the most important elements of the microscope and should be kept meticulously clean, especially the front lens surfaces.
Each objective features engraved numbers and symbols e.g., Plan-Neofluar 20x/0.50; ∞/0.17, which signify:

20x	=	(individual) magnification,
0.50	=	numerical aperture,
∞	=	infinite image distance,
0.17	=	the cover glass thickness in mm, for which the objective is computed.

The (individual) magnification multiplied by the eyepiece magnification (generally 10x) results in the microscope magnification.
The numerical aperture multiplied by 1000 (500 in this example) is the highest useful magnification; no more details will be revealed above this value. The numerical aperture is important in darkfield illumination for the selection of the darkfield diaphragms.

15



The symbol ∞ is to remind the user that these objectives cannot be used on microscopes with objectives engraved with the number 160.
The strict observation of the cover glass thickness 0.17 is the more important the higher the numerical aperture of the objective. Certain objectives are adjustable to different cover glass thicknesses (correction mounts): by means of a high-contrast specimen feature the user finds out in which position of the correction mount he will obtain optimum sharpness (re-focusing will always be necessary). Immersion objectives are insensitive to differences in the cover glass thickness.
Because of the short working distance, objectives 20x and higher have spring mounts to protect the specimen. To prevent specimen contamination by immersion oil when turning the nosepiece, immersion objectives are lockable in the upper position of the spring mount by a clockwise turn (don't forget to disengage them from this "lock-in" position!).

Immersion oil from a plastic oiler replaces the air between cover glass and immersion objective. Some experience is needed to obtain a bubble-free layer. There are microscopists who turn in the objective from the side into the oil drop on the cover glass, others prefer lowering the objective from "lock-in" position of the spring mount. But the exit pupil should always be controlled, preferably using a centering telescope **6.16**, which will instantly reveal any bubbles. If the bubbles have not disappeared after turning in the objective several times, clean the specimen and repeat the procedure.

6.2 Nosepiece, rigidly mounted on the microscope stand.

If the microscope is suitable for DIC, the knurled ring of the nosepiece 5x features
6.3 Slots for
6.4 DIC sliders which must snap in when inserted (designation face up!) (see also DIC adjustment on page 23). If you do not work in DIC, the DIC sliders can be left in the slots as dust covers, provided the polarizer under the condenser is swung out.

The sliders for neutral density filters N 0.08, N 0.15, N 0.30 and N 0.50 can be inserted in **6.3**. This allows the compensation of brightness differences within one series of objectives, e.g. in TV microscopy or microflash photography (see also page 31).

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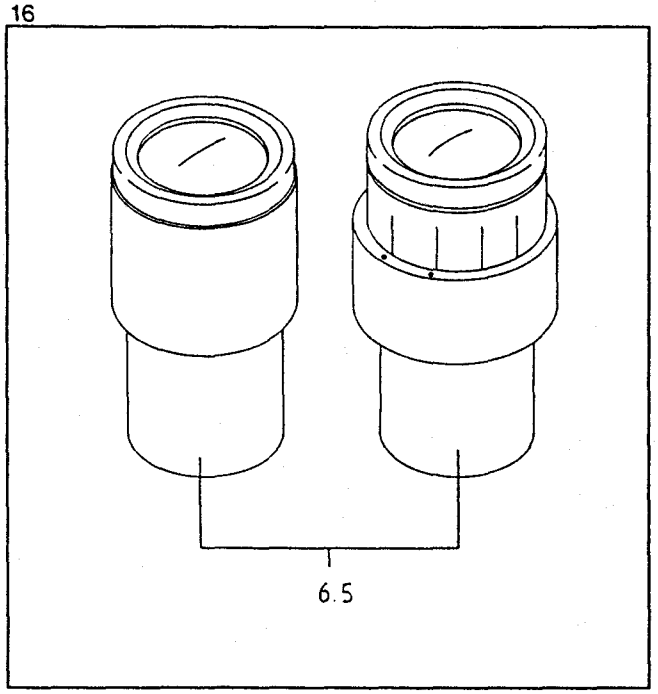

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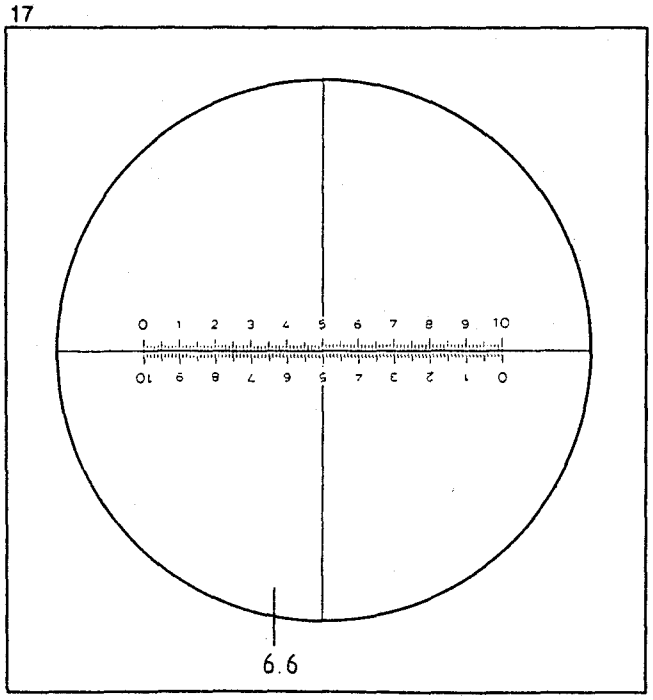

6.5 Eyepieces (10x magnification and field-of-view number 20) produce angular fields of 43°, are ideal for eyeglass wearers (Br), and feature exchangeable, soft rubber rings to protect eyeglasses (folding eyecups are available under ordering number 44 48 01). One eyepiece is a focusing eyepiece (foc). If your eyes have different powers, or for microscopy without eyeglasses, proceed as follows:

- Look through the fixed eyepiece with the less emmetropic eye and focus on the specimen.
- Leave the microscope adjustment unchanged, but re-focus with the eyelens of the focusing eyepiece for the more emmetropic eye, until the focus is the same for both eyes.

This adjustment is facilitated by a screw which engages a notch in the eyepiece tube and thus secures the eyepiece position, which is important especially for the use of reticles.

Eyeglass wearers who work without eyeglasses may experience unsatisfactory objective parfocalization. Eyeglasses with a "cylinder" must be kept on for microscopic work.

6.6 Reticles are used in the eyepiece diaphragm plane for measurement or to outline the photographic format of an attached micrscope camera. They fit only in focusing eyepieces (foc); they bring about a slight image displacement which is considered on the diopter scale of these eyepieces: the zero position is indicated by a red dot. Because of the high demands on cleanliness and exact alignment, reticles should be exchanged by specialists. (The lower part of the eyepiece can be unscrewed; the scale-bearing surface of the reticle must face down.)

6.7 Optovar slider D (45 14 90) with the factors 1x (middle), 1.25x and 1.6x for quick magnification change is inserted in
6.8 if spring pin
6.9 was pulled out which provides for the limit stops.
6.10 Dust covers on both sides of the microscope stand to protect the Optovar slider.
6.11 Lever to set the Optovar factor which is read in the right (factors 1 and 1.25) or left window (factor 1.6).
For retrofitting of the Optovar slider and the dust covers see page 27.
6.12 Slot for insertion of fixed analyzer slider D (43 36 05) (for DIC or Pol contrasting), auxiliary objects and compensators.

Fixed polarizer with rotatable lambda plate (445226)
consisting of upper carrier **6.19** with λ-plate rotatable through 90° and lower carrier **6.20** with fixed polarizer. This unit can be screwed to the condenser carrier from below.
The intensity of the "Red I" color contrast can be set by rotating the λ-plate. "Red I" is most intensive in the position 45° and becomes ineffective in the stop positions 0° and 90°. The analyser must also be inserted into the light path.

If a microscope camera is mounted on an Axioskop microscope equipped for DIC or Pol contrast, insert the quartz depolarizer (45 36 77) in the tube port of the microscope (see instructions for the microscope camera).

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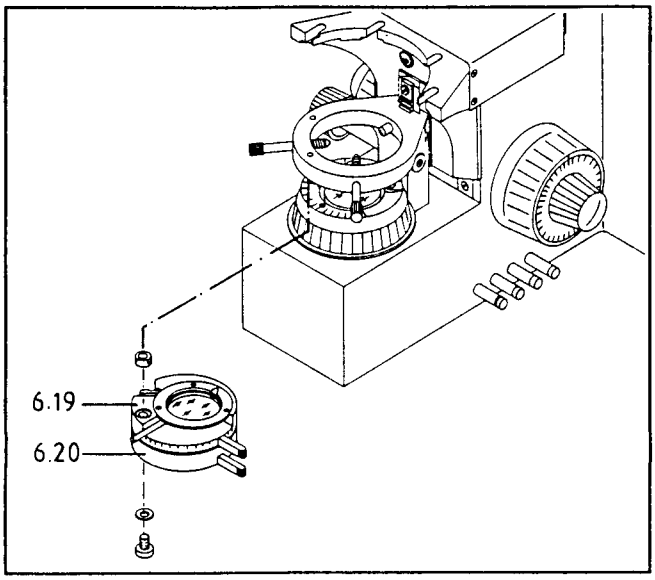

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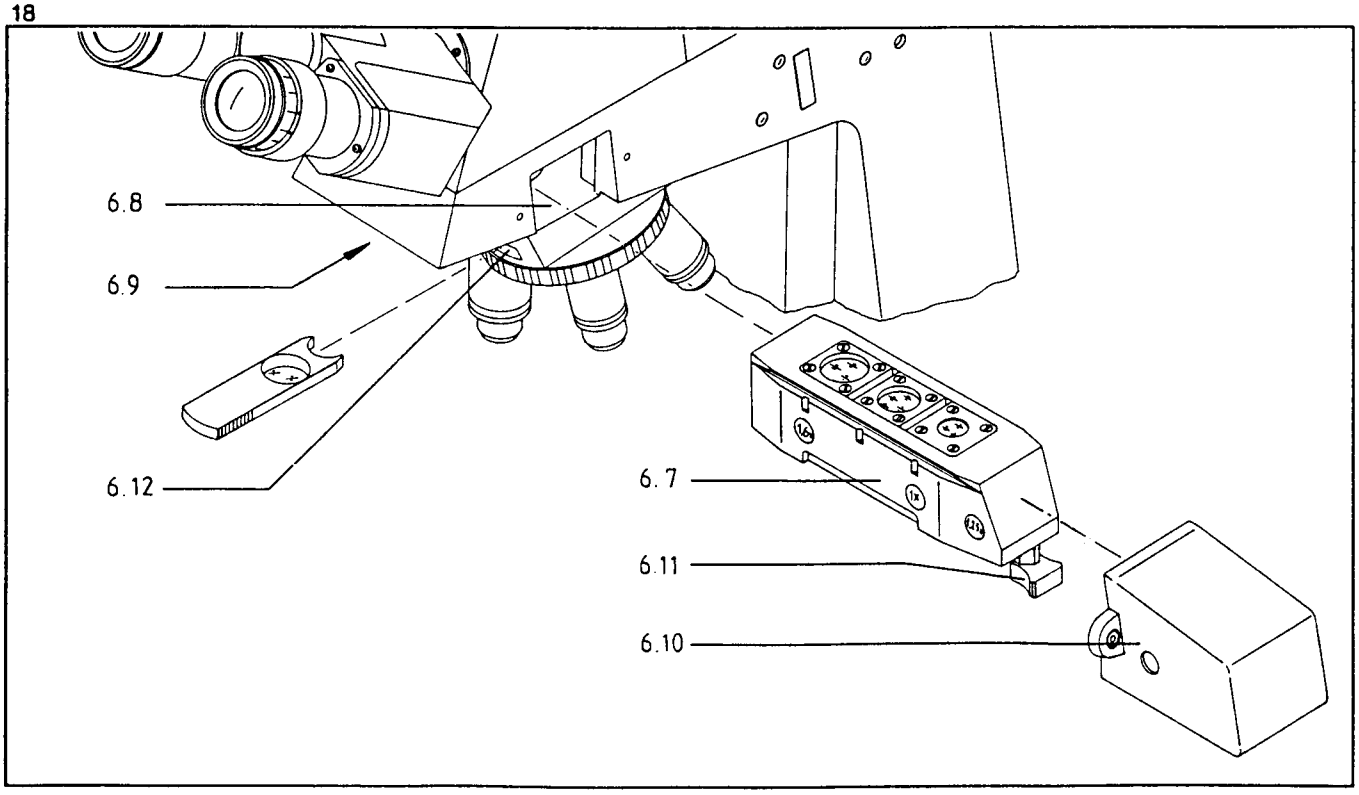

Any of the following tubes is suited for the Axioskop microscope:

6.13 Binocular tube 30°/20 (45 29 05),
6.14 Binocular phototube 30°/20 (45 29 12) with a fixed beam splitting ratio of 50% upwards to the camera and 50% for observation,
6.15 Binocular phototube 30°/20 with sliding prism (45 29 10); two positions can be adjusted using the knob to the right of the pushrod:
pushrod slid in - 100% of the light for observation
pushrod pulled out - 30% for observation and 70% to the camera.
This tube is also available with integrated 1x, 3 T-CTV for TV cameras with ENG bayonet (45 29 40).

The tube also contains an eyepiece shutter which is open in position "observation". In position "camera" the shutter can be moved in or out with a pushrod **6.18**.

Symbols on a label refer the user to the different switch positions of the phototube with eyepiece shutter.

All tubes have a viewing angle of 30°. PDs between 55 and 75mm are adjustable by moving the tube halves in or out. The phototubes feature reticle erection (accurate to 1°).

A photomicrographic or a TV camera can be mounted on the upper tube port. They require:
adapter (45 29 96) for MC 80 and for MC 100 microscope cameras,
adapter (45 29 95) for TV cameras with C-mount,
adapter (45 29 94) for TV cameras with 3T CTV (ENG- bayonet).

6.16 Centering telescope (44 48 30) for convenient observation of the objective pupil, especially for phase-contrast centration, inserted in either tube instead of the eyepiece.

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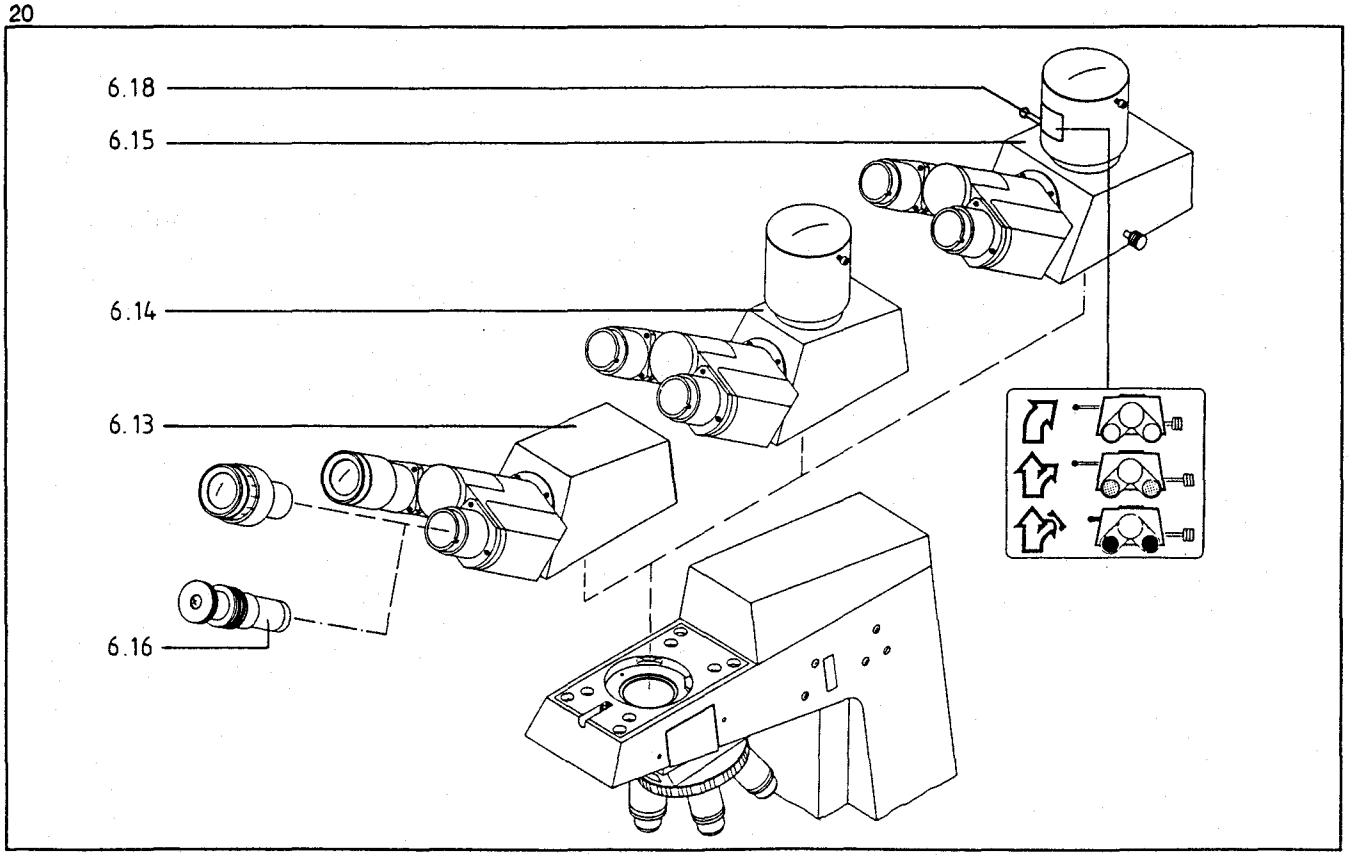

7.0 Fluorescence equipment

The fluorescence version of your microscope includes reflected-light equipment Fl (44 63 60).
Further components are:

7.1 Fluorescence illuminator, including as standard equipment a collector and an HBO 50 mercury lamp which is supplied from a separate power supply (39 26 42).

7.2 Slider marked L for left and R for right, which either interrupts the illuminating beam path (slid in all the way from the left), brings a BG 38 red-attenuating filter in the beam path, which eliminates disturbing IR light (middle position), or provides free aperture (slid in all the way from the right). An additional 18mm dia. exciter filter can be inserted in the free aperture of the slider.

A KG 1 heat-reflecting filter in the stand is invisible from the outside, but does not interfere with UV excitation.

7.3 Lever for luminous field diaphragm
7.4 Centering screws to the left and right on the microscope stand for operation of the luminous field diaphragm.

7.5 Reflector slider 3 Fl inserted in place of the Optovar magnification changer **6.7** in brightfield. Like the Optovar it has three apertures: the central one is generally left free for brightfield or phase contrast observation, the others accept suitable exciter filter/chromatic beam splitter/barrier filter sets. For more details see pages 24 and 28.

7.6 Handle to slide in the selected filter set; display in the right (position I and II) or left window (III).

If the fluorescence reflector 3 Fl is used, fix
7.7 dust covers on both sides of the microscope with 2 screws (tighten the screws alternately), to protect the projecting reflector part against dust and fingerprints.

7.8 Reflector slider FITC (48 79 69) with a filter set for blue excitation (left) and a free aperture (right) for brightfield or phase contrast observation, which can be inserted instead of the reflector slider 3 Fl.
The reflector slider FITC is moved in and out by pressing the outer surfaces.
The designation "Fl 09" is visible on the right side of the slider if the filter set is in the beam path.
Furthermore, the reflector slider 4FL (446425) with suitable protection covers (451458) is available.

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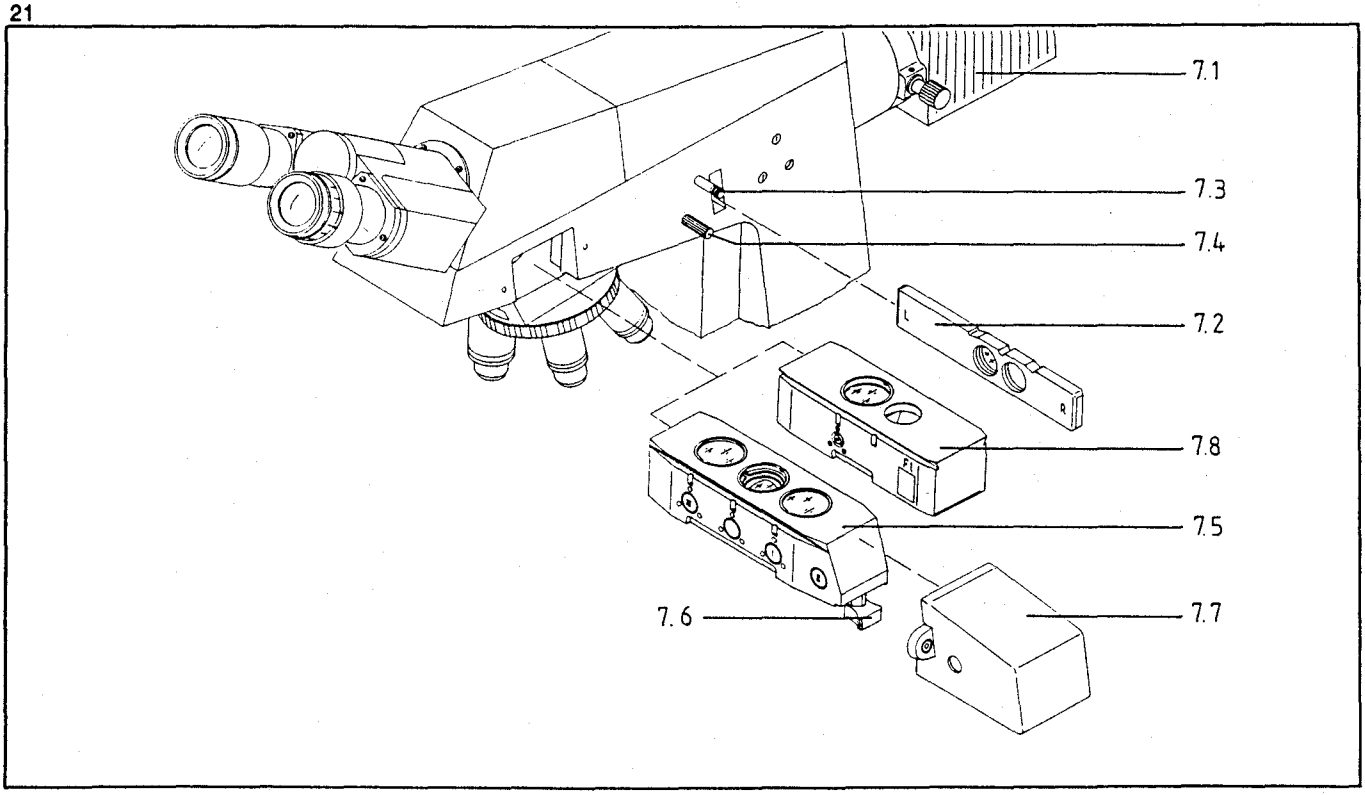

Fluorescence illuminator

The HBO/XBO lamp housing has the following features:

1 Knob for collector adjustment.
2 Vertical lamp adjustment.
3 Lateral lamp adjustment.
4 Vertical adjustment of reflector image.
5 Lateral adjustment of reflector image.
6 Focusing of reflector image.

To center the HBO 50, proceed as follows:

- Put on protective eyewear, e.g. sunglasses, to prevent your eyes from being damaged by UV radiation when the ignited lamp is adjusted.
- Remove switched-on illuminator carefully from the microscope Use screwdriver SW 3 to loosen clamping screw.
- Do not look into the collector opening when manipulating the ignited lamp. Watch the light spot to be adjusted only as long as absolutely necessary in order to avoid damage to the eye by reflecting UV radiation.

- Use knob **1** for collector adjustment to image the brighter of the two light arc images in focus on a wall approx. 3m away.
- Center cathode spot image using SW 3 Allen wrench on adjusting screws **2** and **3**.
- Use screw **6** to refocus the reflector image, if necessary, and take care that lamp image and reflector image have the same size.
- Use adjusting screws **4** and **5** (marked red) to adjust the reflector image and the lamp image side by side; the two images must not overlap (see Fig. 23).

- Attach illuminator to the microscope again. Set slider of FI reflected-light illumination to free light path and set reflector slider to blue excitation (see microscope instructions).

- Unscrew an objective and check the image of the light source on a sheet of paper placed in the object plane (on the microscope stage).
- Make corrections with knob **1** and adjusting aids **2 - 6**. Screw in objective again.

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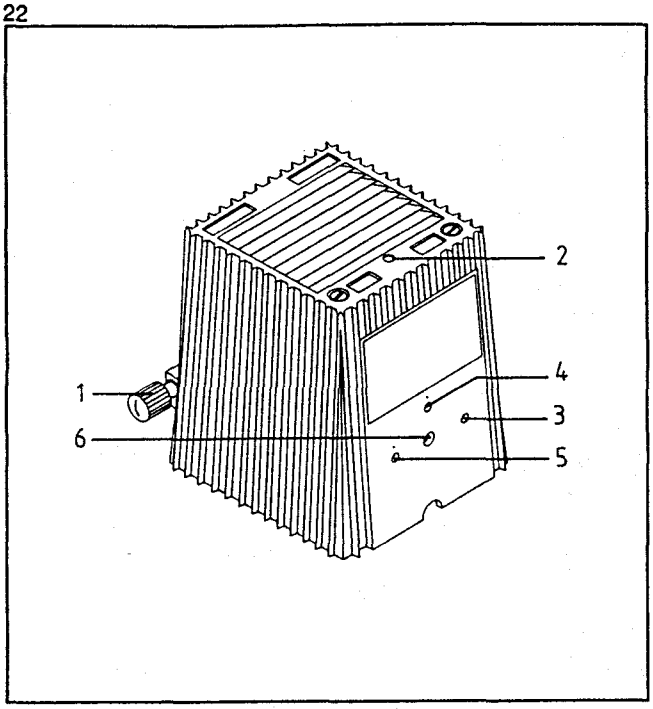

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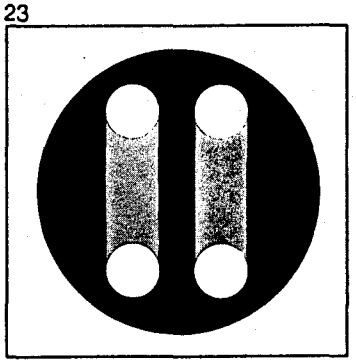

The HBO 50 mercury short arc lamp must be exchanged after expiration of the mean lifetime of 100 hrs. Its illuminance decreases in the course of many hours of use so that homogeneous illumination of the object field can no longer be guaranteed. There is also a danger of explosion. The remaining lifetime can be read off on the power supply unit.

For more details and specially the important safety provisions we refer to the manual
G 42-160 Microscope lamp HBO 50 for fluorescence

It is applied mainly to enhance the contrast of unstained specimens.

Necessary equipment

- Objectives (4) designated Ph, which are equallly well suited for brightfield microscopy.
- A turret condenser (5) equipped with phase stops.

Necessary additional adjustments

The phase rings in the objectives are of different sizes and indicated on the objective (4) by Ph 1, Ph 2 and Ph 3.
The turret (5) bears the same designations - Ph1, Ph2 and Ph3 for combination with the suitable objective. Condenser turrets with 2 or 3 Ph positions are available (**5.16** and **5.24**).

Perfect phase contrast is produced if the dark ring in the objective and the bright ring in the condenser exactly coincide. To control the adjustment, insert the centering telescope (3), and focus with the eyelens (2) of the telescope held by its knurled ring (1).
(Without centering telescope the adjustment is controlled like the condenser diaphragm adjustment (see page 5), with the eyepiece removed.)
The two condensers 0.9 Ph (44 52 36) and 0.9 Ph DIC (44 52 41) feature 2 centering screws (7).

Centering of Ph condenser rings

The Ph condenser rings are precentered in the factory. In case they are decentered, proceed as follows:

- insert the two keys supplied into centering screws (7) and
- turn the keys alternatively until the bright Ph condenser ring fully coincides with the Ph objective ring (Fig. 25).

Should you have difficulties in inserting the keys into the centering screws when the condenser is inserted, we would recommend you to proceed as follows:

- lower the condenser in the condenser carrier,
- insert the two keys into centering screws (7),
- move the condenser upward into working position and center the Ph condenser rings as described before.

Centering of the Ph 1, Ph 2 and Ph 3 condenser rings must be performed only once; it is then maintained for future work.

The image contrast may be enchanced by a green filter in the filter magazine (8), in the color glass carrier or on the luminous field diaphragm.

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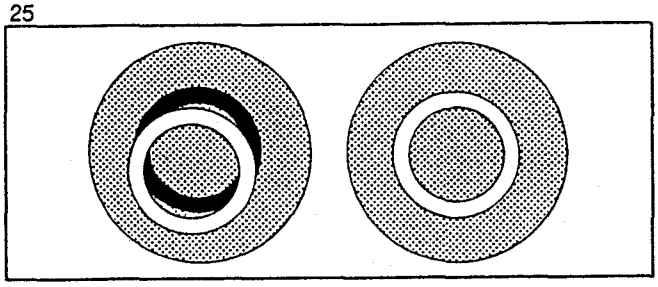

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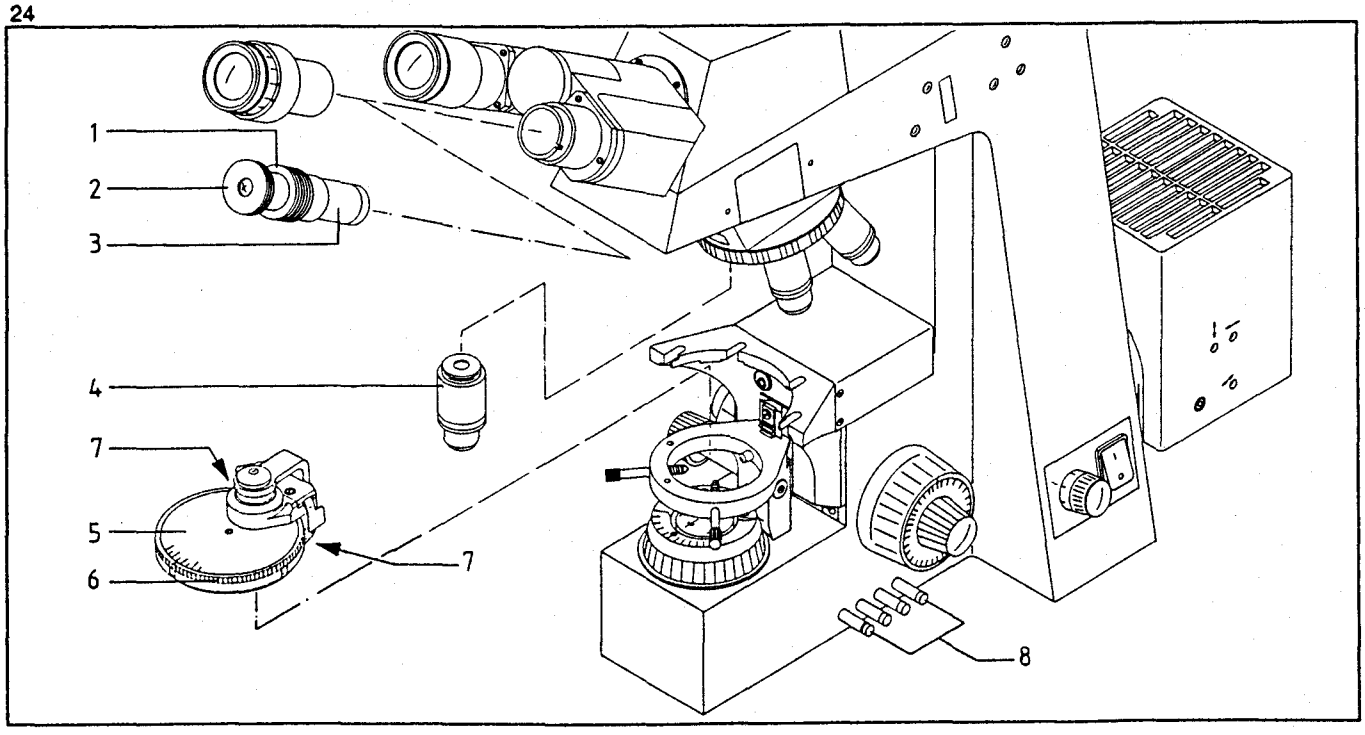

Necessary equipment
- Plan-Neofluar objectives for UV excitation, otherwise no special objectives required.
- Special incident-light illuminator (see page 18).

Procedure
- Set the reflector slider 3 FI (1) to middle position (free aperture) and with the lower illuminator with halogen lamp adjust the selected specimen feature in brightfield or transmitted-light phase contrast. Switch on the mercury lamp but block its light path with slider (3).
- Switch off the transmitted-light illumination, or at least reduce its brightness considerably, remove all filters in the filter magazine from the beam path, and, depending on the type of excitation, move the reflector slider to the left or right position. Open the light path with (3).
- Closing an aperture diaphragm in the illuminating beam path would reduce the brightness of the image in fluorescence observation.

Only a luminous field diaphragm is, therefore, provided. Close it with lever (5) until it becomes visible in the image. Then center with (4) and open it until it has disappeared from the field of view.

Special notes
Start the adjustment for fluorecence microscopy with a 20x objective and a strongly fluorecent specimen (suitable specimens are available but may also be prepared by the user; a specimen of spread anthracene crystals is quite popular). You may even use the specimen label for a check of the illumination.

The reflector slider contains several filter sets. Each set comprises 25mm dia. exciter and barrier filters, enclosing a 26 x 26mm chromatic beam splitter. For more information about filters and their exchange see page 28.

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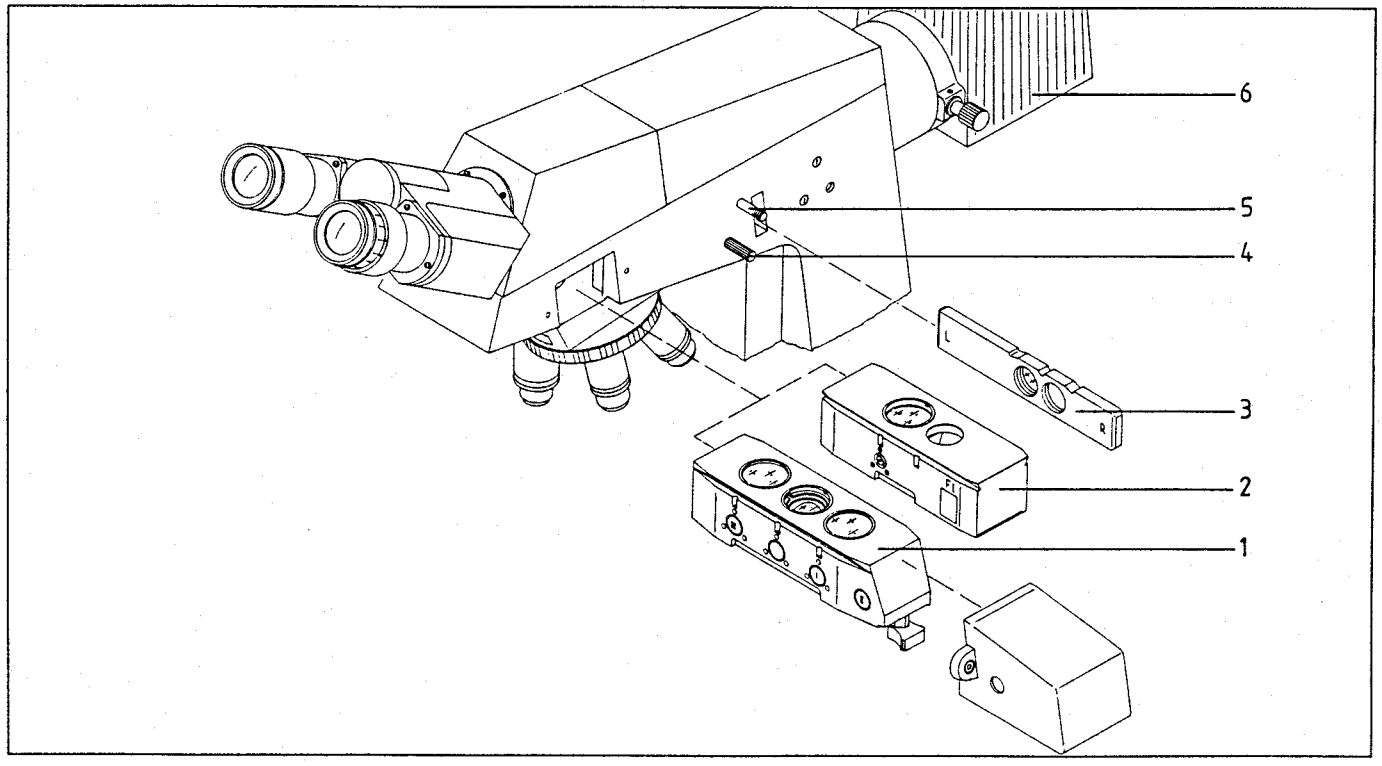

It is applied

- to study exceptionally small objects or object features such as treponemas, spirochaetae, bacteria, flagellates, etc., or emulsions, if phase contrast does not supply sufficient contrast;
- if the specific colors of natural (unstained) objects are well visible (living organisms in water like algae, unicellular organisms, lower animals).

Necessary equipment

- Special objectives with integral iris diaphragm only for higher magnifications, but
- always a condenser with central stop and a numerical aperture which is higher than that of the objective used.

For further details see the opposite table.

Necessary adjustments

- Adjust the illumination as for brightfield. The luminous field diaphragm must be imaged and centered.
- Check objective pupil for perfect darkness. Objectives with iris diaphragm have too high apertures for darkfield. They must be stopped down with the iris as far as the limit aperture. The most important criterion is, of course, the absolutely dark background of the eyepiece image, which may be influenced by the position of the luminous field diaphragm, especially towards the edge of the field of view.

Centering the darkfield diaphragm:

- Adjust Köhler illumination with the condenser in position H (see page 5).
- Switch the condenser to darkfield illumination.
- Open the luminous field diaphragm. Remove the eyepiece from the tube. Check the objective exit pupil. If it does not display uniform darkness, center the darkfield diaphragm D with the Allen wrench SW 0.9 at **5.30** (see p. 13). Insert eyepiece in the tube and observe the object field. Correct any reflections by slightly varying the height of the condenser.

A similar procedure applies for other darkfield condensers.

Special notes

The cleanliness of the specimens is much more important in darkfield than in other methods; grease films (fingerprints) are especially disturbing because they light up the background. Pre-centering with a low-power objective eases darkfield adjustment. The luminous field becomes visible only where particles light up, but wide areas of a specimen may be "blank". We recommend to use a specimen with uniform feature distribution for the initial adjustment, e.g. a blood smear.

Darkfield illumination
Various possible aperture ranges of the objectives

Condenser Illumination aperture	Aperture of suitable objectives
Condenser 0.9 H D Ph with swing-in front lens in position Ph 3 0.44 - 0.56	< 0.4
Condenser 0.9 H D Ph with swing-in front lens Darkfield diaphragm 0.75 - 0.90 (44 52 99)	0.5 - 0.75
Dry darkfield condenser 0.7 - 0.85 (46 55 06) on condenser holder Z (44 52 15)	0.4 - 0.6
Dry darkfield condenser 0.8 - 0.95 (46 55 05) on condenser holder Z (44 52 15)	0.6 - 0.75
Ultra condenser 1.2 - 1.4 (46 55 00) on condenser holder Z (44 52 15)	0.75 - 1.0

Darkfield illuminator (1) (44 52 14) for low magnifications

Adjustment of optimum darkfield illumination

- Insert illuminator (1) in condenser carrier; spring pin (3) must engage the alignment notch.
- With condenser knob move illuminator beneath stage carrier.
- Turn in 1.25x objective.
- Load scattering auxiliary specimen (paper strip) and focus.
- Close luminous field diaphragm (5). Produce homogeneous vignetting at upper and lower edge of the field of view. This changes the inclination of the spherical mirror. Produce right/left symmetry using the two centering screws (4) of the condenser carrier. The remaining vignetting in the upper and lower areas of the field of view is unavoidable for technical reasons. But the photographic field is homogeneously illuminated.
- Load specimen. Large specimen slides and cover glasses are most suitable, because scattering edges lie far outside the object field.
- If the brightness of the image is too low, the diffusing screen (6) can be swung out. Opening the luminous field diaphragm (5) will also increase the brightness of the specimen, but also the number of reflections outside the photographic field.
- When changing to the next higher objective magnification of 2.5x, vary inclination of mirror using screw (2) until the field of view is illuminated without reflections. Adjust the luminous field diaphragm until the darkness of the image background is optimized. The adjustment of the luminous field with constant mirror adjustment is sufficient at objective magnifications up to 20x.

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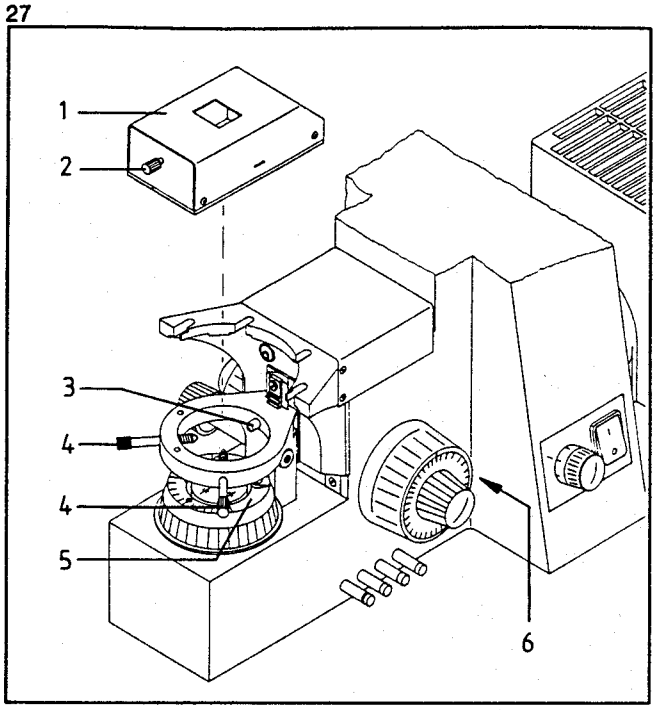


It is applied, for instance, if a specimen is too thick for examination in phase contast and specimen layers outside the focal plane impair the brilliance of the image, or if the halo which is typical of phase contrast interferes with the observation of small features.

Necessary equipment

- As a rule Neofluar objectives, and for most exacting demands, Plan-Neofluar Pol objectives.
- A special nosepiece (5) with slots (2) for
- a special DIC slider (3) for each objective; on its top surface it bears magnification and aperture of the objective for which it is intended.
- Achromatic-aplanatic condenser 0.9 Ph DIC with swing-in front lens (44 52 41) (6) or achromatic-aplanatic condenser system (7) with front lens 0.63 Pol or 1.4 Pol, each with suitable DIC prism (see opposite table).
- A polarizer (8) which is swung in under the condenser.
- An analyzer (4) slid into slot (1).
- With microscope camera, a quartz depolarizer which may be firmly mounted in the tube port.

Necessary additional adjustments

The condenser system contains only 1 DIC prism, which limits microscopy in DIC to the aperture range of this prism (for exchange of the prism see page 26).

Set the corresponding DIC click stop on the condenser 0.9 Ph DIC with swing-in front lens.

Combinations condenser - DIC prism - objective aperture

Condenser	DIC prism	Objective NA
Condenser system 0,32 Pol with front lens 0.63 Pol	NA 0.3 - 0.4/0.63 (43 44 10)	0.3 - 0.4
Condenser 0.9 Ph DIC with swing-in front lens	NA 0.3 - 0.4/0.9 (44 52 73)	0.3 - 0.4
Condenser system 0.32 Pol with front lens 1.4 Pol	NA 0.5-1.4/1.4 (44 52 94)	0.5 - 1.4
Condenser 0.9 Ph DIC with swing-in front lens	NA 0.5-1.4/0.9 (44 52 74)	0.5 - 1.4

- In differential interference contrast you should use an iris diaphragm which you open completely at first and, as last step of the adjustment, close slightly to enhance the contrast.

- The DIC sliders (3) in the nosepiece are provided with knurled screws to optimize the contrast.
If the slider is moved symmetrically from its center position, specimen features can be visualized 3-dimensionally so that they appear either raised or lowered.

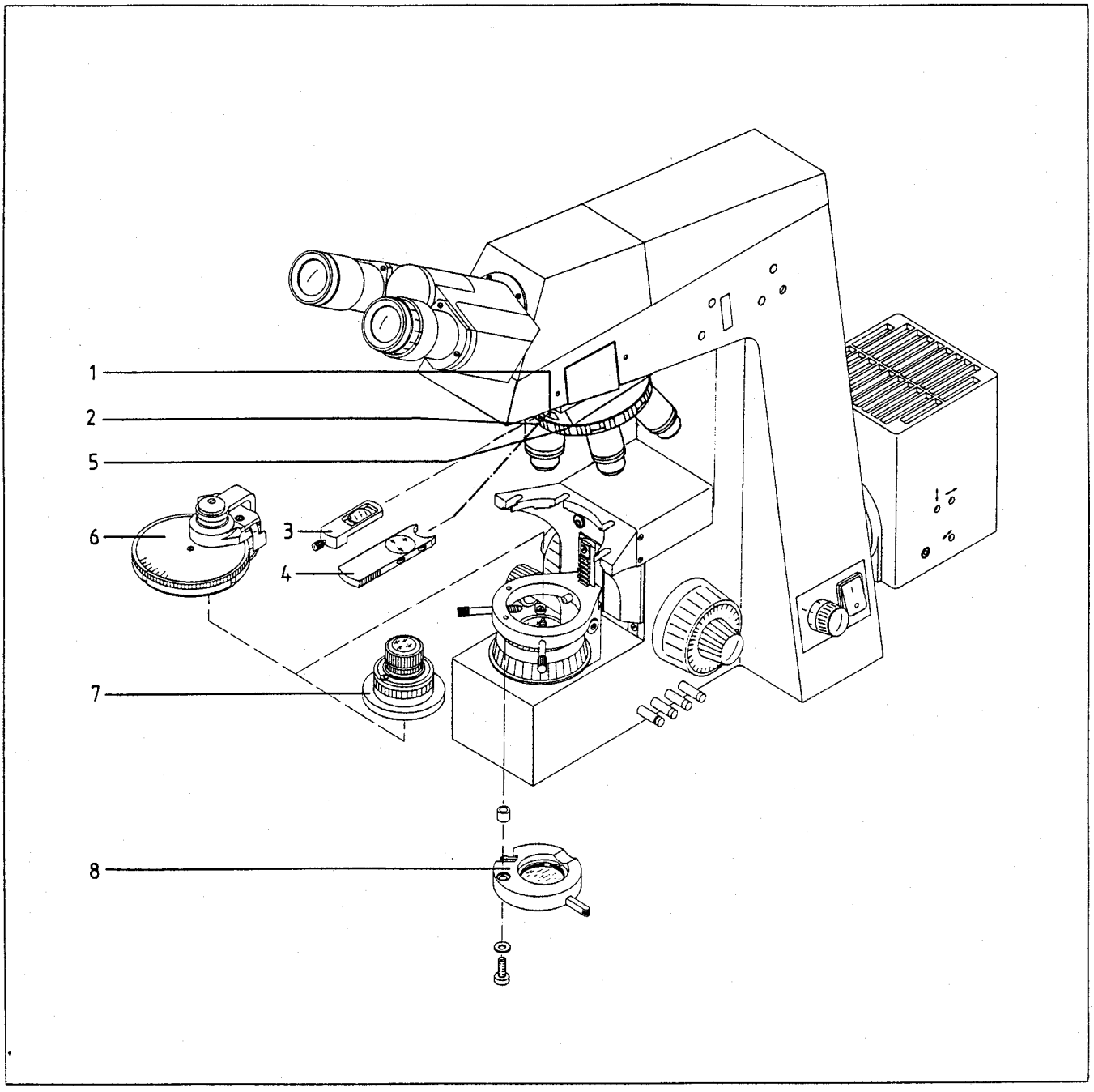
Special notes

The contrast is produced in DIC by a (pseudo) relief. The contrast of linear structures is lower if they are oriented in "light-shadow" direction, but highest if the orientation is at right angles to this direction. The possibility to rotate the specimen is, therefore, of great advantage. Please refer to page 10, **4.6** for the use of the mechanical stage as rotary stage.

The luminous field and aperture diaphragms should not be opened wider than required for Köhler illumination to ensure reflex-free illumination (see page 5).

As DIC uses polarized light, "optically active" elements between polarizer and analyzer would be disturbing, e.g. mica plates which are sometimes used for histological sections, or plexiglass culture dishes with plastic bottoms (dishes with glass bottoms are available). The use of such elements may impair the performance.

28



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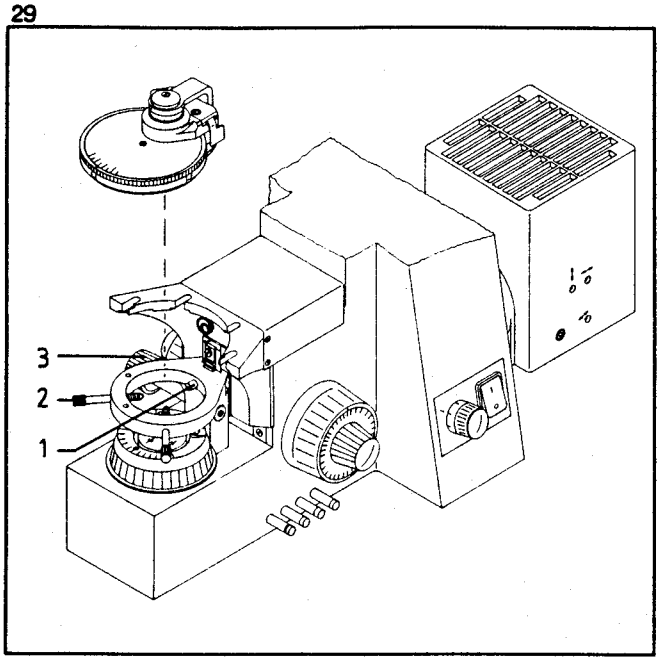

Should minor changes be necessary on your microscope, and no service technician available, the following hints may be helpful.

Condenser exchange

Exchange of a brightfield condenser for a condenser system for brightfield/phase contast/DIC: lower the condenser with (3) as far as possible, turn the front centering screws (2) back by a small amount, press the condenser backwards against the spring pin of the condenser carrier, and lift it out in front. Insert the new condenser accordingly. Its notch must engage spring pin (1) to ensure exact fitting.

When inserting the condenser system (Fig. 29) with DIC prism, make sure that the white dot on the condenser bottom points towards the user.

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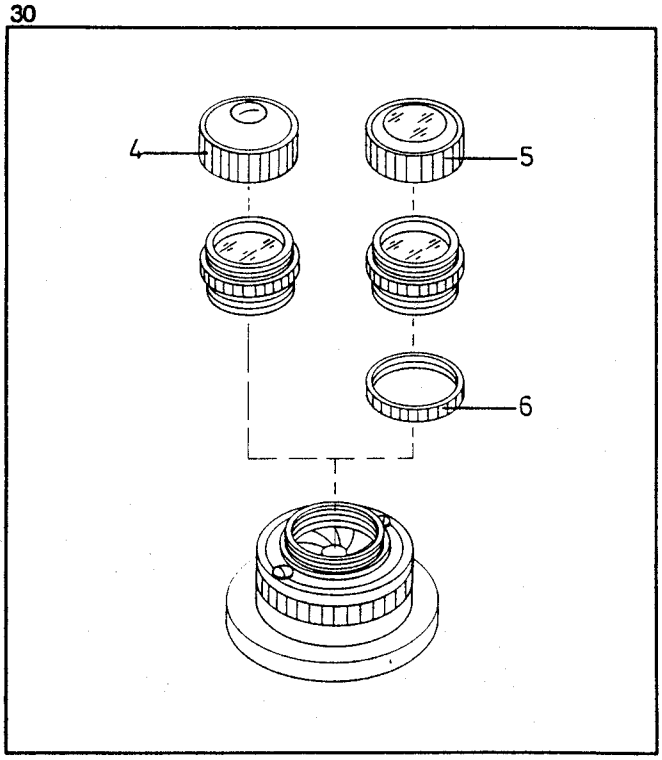

Exchange of condenser front lens

It is possible to equip the condenser system 0.32 Pol with a front lens 1.4 Pol (4) or 0.63 Pol (5) for long back focal distance (7mm in air, 11mm in glass). Both front lenses are screwed on the condenser system 0.32. A spacer ring 0.63 (6) is required for the front lens 0.63 Pol between condenser body and condenser part 0.32.

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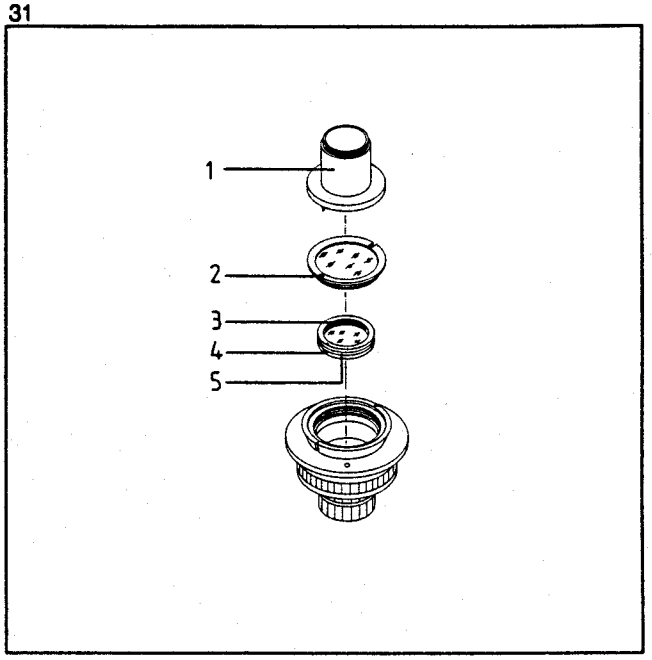

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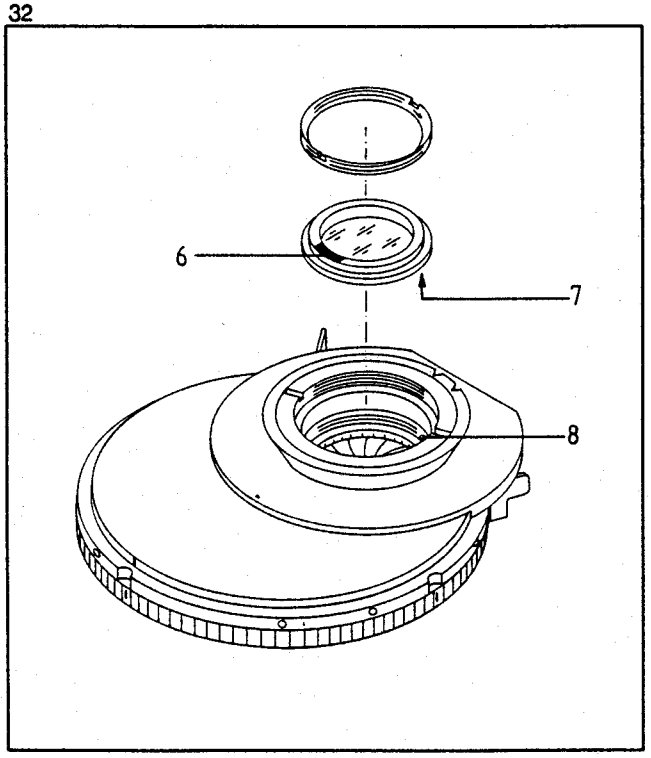

Exchange of DIC prisms in condensers

Which prism is used depends on the objective and the numerical aperture of the condenser system (see table on page 24).

For exchange of an achromatic-aplanatic condenser system (44 52 45-9902) remove auxiliary lens (2) with supplied tool (1). Then screw the external thread of the tool into prism mount (3). Pull out prism (5) which is held by an annular notch (4) of the prism mount and a wire loop in the holder. A new prism is correctly inserted only if notch (4) marked by a dot and the corresponding pin of the holder engage. Check for correct flat seating to prevent mechanical and optical interferences.

The achromatic-aplanatic condenser 0.9 Ph DIC with swing-in front lens (44 52 41) is provided with two click stops for DIC prisms. An exchange is hardly ever necessary.
To insert a DIC prism in the turret: hold the condenser upside down
(see Fig. 32), and turn an aperture with iris diaphragm into the optical axis of the condenser; the index will point to "DIC".
Put a prism in the aperture; the white mark on the mount should point to 6 o'clock. Move the prism mount slightly to make sure that the pin has engaged the corresponding borehole of the aperture.
Put on the retaining ring with its two grooves on top and screw it in with auxiliary tool, which secures the prism.

Phase stops Ph, darkfield diaphragm D (44 52 99) or auxiliary lens 1.25x (44 52 65)
They are fitted in the two centerable apertures. This also applies to the achromatic-aplanatic condenser 0.9 H, D, Ph (44 52 36) (see page 13).
Turn the condenser aperture to the relevant position. Unscrew the retaining ring. The polished glass surface of the diaphragm or the convex surface of the auxiliary lens should be on top. If the diaphragm/lens cannot be secured by the retaining ring because the mount follows up, fix the mount with a centering screw.
For the centration of the phase stop Ph, see page 20.

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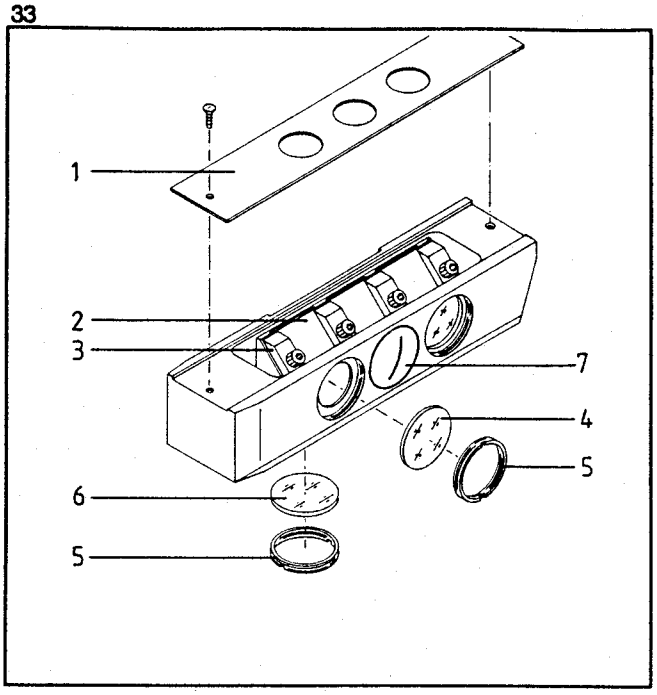

Fluorescence reflector 3 FL

Exchange of exciter filter (4), barrier filter (6) and chromatic beam splitter (2): unscrew retaining rings (5) for an exchange of the built-in filter sets. Remove base plate (1); the plate carrying the chromatic beam splitters (2) is accessible. This plate rests on a spring mask and should not be touched. Straps (3) need not be entirely removed; they must only be loosened for an exchange of the beam splitters on the mask.
The middle position of the reflector slider is normally a free aperture intended for observation in transmitted light.
The holder for exciter filter is then covered to prevent straylight from interfering with transmitted-light observation.
Cover (7) must be removed if a filter set is to be inserted in the middle position of the reflector slider.

Filter sets

Type of excitation	Filter set	Exciter filter	Chroma-tic beam splitter	Barrierfilter filter
UV-G 365	48 79 02	G 365	FT 395	LP 420
Blue-violet G 436	48 79 07	G 436	FT 510	LP 520
UV-H 365	48 79 01	BP 365/12	FT 395	LP 397
Blue-violet H 436	48 79 06	BP 436/10	FT 460	LP 470
Blue H 485	48 79 16	BP 485/20	FT 510	LP 520
Blue H 485 SB	48 79 17	BP 485/20	FT 510	BP 515-565
Green H 546	48 79 15	BP 546/12	FT 580	LP 590
UV-violet 390-420	48 79 18	BP 390-420	FT 425	LP 540

Type of excitation	Filter set	Exciter filter	Chroma-tic beam splitter	Barrier filter
Blue-violet 395-440	48 79 05	BP 395-440	FT 460	LP 470
Blue 450-490	48 79 09	BP 450-490	FT 510	LP 520
Blue 450-490 SB	48 79 10	BP 450-490	FT 510	BP 515-565
Green 510-560	48 79 14	LP 510 - KP560	FT 580	LP 590
Green 530-585	48 79 00	BP 530-585	FT 600	LP 615
Green H 546	48 79 20	BP 546/12	FT 560	BP 575-640
FURA-2 UV 340+380	48 79 21	BP 340/10* BP 380/10*	FT 395	BP 500-530

*Filter 18 mm dia.

Tube

For tube exchange loosen the Allen screw (1) and take off the tube. Put on the new tube and secure it. (The Allen screw must be unscrewed so far that it is no longer visible inside.)

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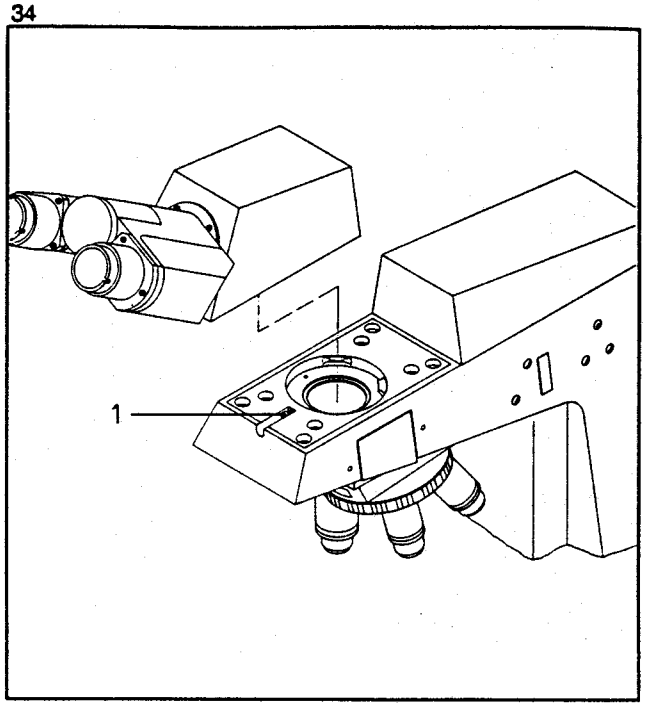

Insertion of the Optovar slider or the reflector slider 3 FI

Pull spring pin (2) and remove blank slider, if any, from slot (3). Fix the dust cover (6) with two screws on the left slot.
Pull spring pin (2) and slide in Optovar slider (4) or reflector slider 3 FI from the right as far as the click stop. Insert the slider dovetails untilted in the guideways of the stand. The handle of the slider (5) should be at the lower right. Fix the right dust cover (6) on the stand by alternately tightening the right and left Allen screw.

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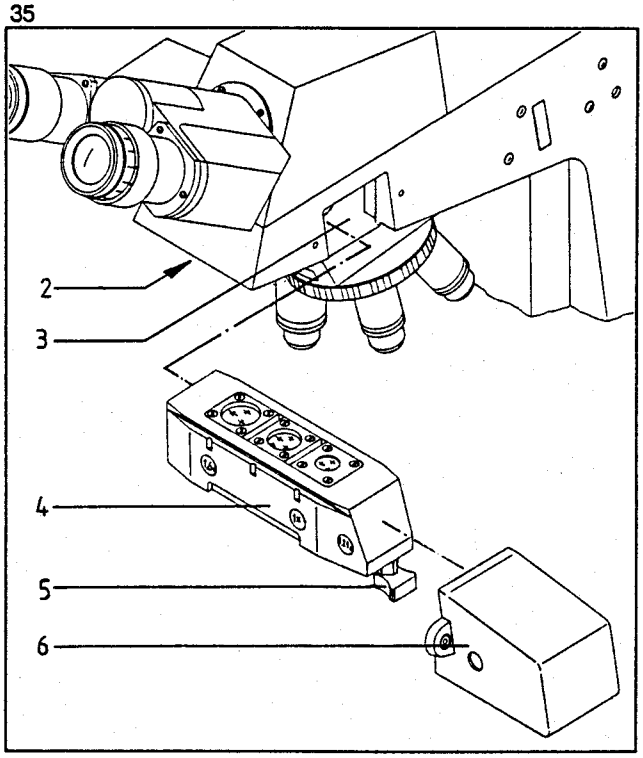

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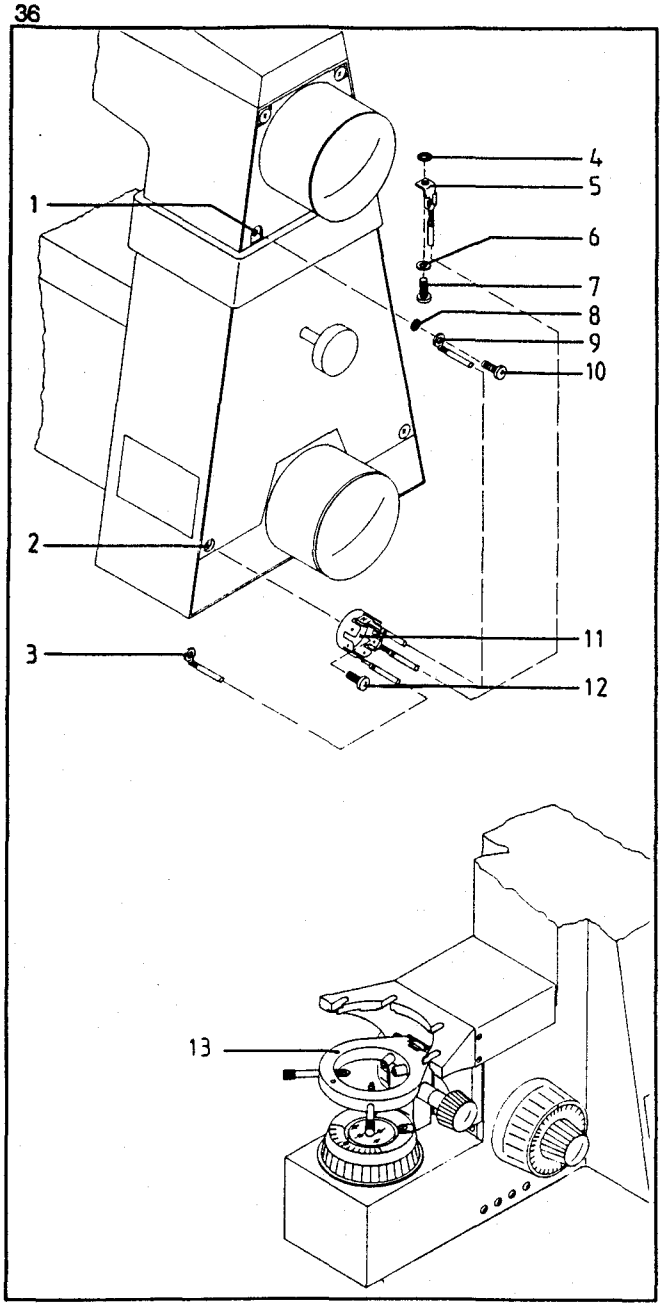

Grounding of the Axioskop FS

Recommended for electro-physiological examinations to ensure *unobstructed current drainage from the specimen.* The grounding of the Axioskop FS (451449) will set the microscope components to earth potential.

Attach grounding connector (11) to the microscope:
Use Philips screw of the lower cover plate on the back of the microscope. The fixation point (2) should preferably be chosen on the bottom right.

Bonding of the lower part of the stand with the upper part:
Attach short cable (9) to the cover plate at (1) with Philips screw (10). Insert sprocket washer (8).

Bonding of the condenser carrier with the stand
Attach connecting piece (5) with washer (6) for the screwhead and sprocket washer (4) for the condenser carrier to the rear drilled hole (13) of the condenser carrier from below. Slide plug-on sleeve of the medium-long cable on the connecting piece.

If the microscope is equipped with a polarizer carrier, the stop bolt can be used for attachment of the connecting piece (5) instead of the screw (6). Washer (4) must be inserted in any case.

Grounding to the microscope:
Attach long cable and cable terminal (3) to an external grounding point with a screw.

Note:
Securely tighten all screws to make the microscope potential-free.

The spare parts are listed below in the order as they appear in the description of the instrument starting on page 6.

1.2 Fuses for the power supply integrated in the stand:
for 230V: T 2 A/H (INR 149.688)
for 115V: T 4 A (INR.144.060)

2.1 42mm dia. heat-reflecting filter (46 78 28), to be inserted in such a way that the surface with higher reflection faces the light source.

6.1 50cc immersion oil in plastic oiler (46 29 58)

7.2 18mm dia. BG 38 red-attenuating filter (46 79 91-9902)

Slider for neutral density filters to compensate the brightness within one series of objectives
(to be inserted in slot **6.3** of the DIC nosepiece, see page 14).

Neutral density filter N 0.08	44 44 90
Neutral density filter N 0.15	44 44 91
Neutral density filter N 0.30	44 44 92
Neutral density filter N 0.50	44 44 93

Power supply in the stand

The power supply is highly stabilized to power fluctuations, provides DC, variable from 3 to 12V and is suitable for demanding photometry.

Input voltage	230 V for 220...240 V 115 V for 100...120 V
Maximum power consumption:	130 VA

Safety regulations:
In compliance with VDE-GS, IEC 1001-1, CSA and UL regulations.
The instrument is radio-screened and short-circuit proof.

Ambient conditions
Use instrument indoors only!

Temperature range	5°C ... max. 40°C
Relative humidity	max. 80%

Avoid condensation!

Instrument type
Safety class I
Contamination type 1
Overvoltage category II

Halogen lamp	**38 00 79-9550**
Lamp voltage	12 V
Power	50 W
Color temperature at 12 V	2800 K*
Luminous flux	950 lm
Mean life	2000 hrs
Luminous surface	3.1x3.1 mm^2

* The built-in conversion filter CB 3, see **1.4**, provides for the right color temperature in the beam path for photomicrography with artificial-light color reversal film which is sensitized for 3200 K.

Mercury short arc lamp HBO 50W/AC	**38 16 19**
Lamp voltage	L1: 39. . .45 V / L2: 34. . .39 V
Lamp current	L1: 1.30 A / L2: 1.45 A
Power	50 W
Line spectrum	
Mean luminous flux	2 000 lm
Mean life	100 hrs
Luminous area	0.3x1 mm^2

L1, L2: HBO 50W/AC lamps are produced in two versions. The lamp type is given on the enclosed label. Set power supply to lamp type L1 or L2.

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The resolution to succeed .

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